Biogeochemical Investigation of Caves within Bahamian Carbonate Platforms

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A thesis submitted to the University of Bristol
in accordance with the requirements of
the degree of Doctor of Philosophy
in the Faculty of Science

1999

CONTAINS

PULLOUTS

Es erhebt sich nun die Frage,
was unter den naturlichen Lebensbedingungen
dieser Organismen ihre Stoffwechsel sind.
Leider laßt sie sich mit voller Bestimmtheit nicht beantworten

NathanSohn, 1902

The question has arisen
What is the form of energy used by these organisms in their natural environment. Unfortunately, this question cannot be answered with certainty.

(NathanSohn, 1902)

Abstract

The Bahamas are thousands of islands stretching along the coast of Florida and south to the tropic of cancer. An aerial view, spectacular as it is, shows only a small part of the islands, a larger part is submerged and discernible only by the abundance of blue dots onshore and in the shallow water around the islands. The blue holes are entrances to an underwater world wherein biology seems to have slowed down to offer a fascinating view of species rarely seen elsewhere, and of rock formations that tell a story of the subterranian world below the islands.

My studies were inspired by curiosity and the desire to make a contribution to the preservation of this unique and irreplaceable window into the past for geologists and biologists alike.

Important questions as to how the caves formed and when and what the role of cave life might have played in the excavation processes are addressed in this thesis.

As a result of these studies bacteria must be considered as possibly active participants in the formation processes after the time when the rising watertable flooded all the Bahamian caves. The layering, often in hypersharp zones of biological material, matches or is perhaps caused by, equally dramatic changes in hydrogen ions and acetate and sulphate concentrations. In both caves, pH profiles were observed that varied sharply over very short distances.

In all three study sites it could be shown that, vertically and horizontally, the geochemical perimeters varied dramatically. Organically mediated processes are a dominant control on dissolution within the fresh, mixing, and saline zone. The combined generation of CO_2 results, known from earlier studies, and H_2S and other bacterially measured activity, supports this finding. Retention of suspended organic matter was directly proportional to the salinity gradient. Elevated levels of DOC, POM, and acetate at the two major density interfaces confirm this finding. In the results, generated from CHN&S methods, wall rock material was found to contain residual carbon for potential microbial use, and large bacterial populations were identified through the SEM method in excess of what was measured within the water column.

Significant lepidocrocite deposits (dimorphose iron oxihydroxide) were identified as a fraction of the cave sediment, and material known as "mung", based on amino-acid analysis, was shown to be proteinaceous. This mung appears to be unique to caves in the Bahamas.

In a sense the cave system is an underground geo-biosphere wherein the peculiar water flow patterns foster a specific, sometimes rich and unusual, fauna and flora that is a treasure for science and a sight for humans to enjoy.

To my parents, Jutta and Christian Schwabe
with all my love

Acknowledgments

In May of 1997, while I was writing this thesis came the news that my best friend, partner and husband, Robert John Palmer, had disappeared into the blue depths of the Red Sea, not to be seen again. Three months following, I lost another friend of seven years, Rob Parker, during a filming expedition in the Bahamas. The year 1997 was a year of trials and tribulations that I never expected to deal with at this stage in my life. Without the enormous help of friends and family throughout the world, I would not have been able, in a timely fashion, to finish this project. Rob Palmer introduced me to the magnificent world of blue holes in the Bahamas and I will never be able to thank him enough for that. It was our life long plan to study and protect these amazing places and although he is gone, the work has been continuing through "The Rob Palmer Blue Holes Foundation", and will do so as long as I am able. I never got to thank you Rob but I hope to do so with the completion of this project in which you helped me so much and in the completion of many more exciting projects yet to come; you were simply the best.

Along with Rob, this project could not have happened without the enormous support from John Parkes. You held this project together and one day I hope to convince you to come dive in my world for a microbial mind blowing experience. Pete Smart and Fiona Whitaker I would like to thank also for your support and help in this project. The geomicrobiology lab crew, you're the best, especially Barry Cragg and Peter Wellsbury. You two were a strong case for not spending to much of your life in a lab. Thanks for keeping things so interesting and teaching me about British humour..I think. Also Pete, thanks for running the HPLC derivatisation method for acetate. For the CHN&S analysis I thank Mark Teece for risking life and limb to help out a poor student and for library research into the archives for the historical information concerning blue holes I thank Traver Shaw. Many thanks also to Dr. Christian Schwabe for running the amino acid analysis on the mung sediments recovered from the cave and Dr. Erika Büllesbach for helping in the final preperations of this thesis.

Phil Chapman, Elaine Belcher, John and Farzzi Bantin, Dick and Fiona Willis, and in the Bahamas, Ben and Judy Rose; thank you all for keeping me off the streets and well fed. For helping me to keep myself financially afloat for nearly a year, I thank you Gerhard Stütting for trusting me enough and going ahead with the filming of the "Mysterious Blue Holes of the Bahamas" despite Rob's death. This film aired April 19th, 1997 on National television in Germany and France. You gave me an opportunity to push myself beyond known limits and personally grow from the experience. The project gave me something to focus on during my darkest hours. I

also thank you for the opportunity to share with millions of other people, what Rob and I loved so much. I would also like to extend a heart felt thanks to Chris Rose for spending weeks and other odd hours with me following Rob's death, convincing me that life was worth living and I would see light again; I see the light now Chris.

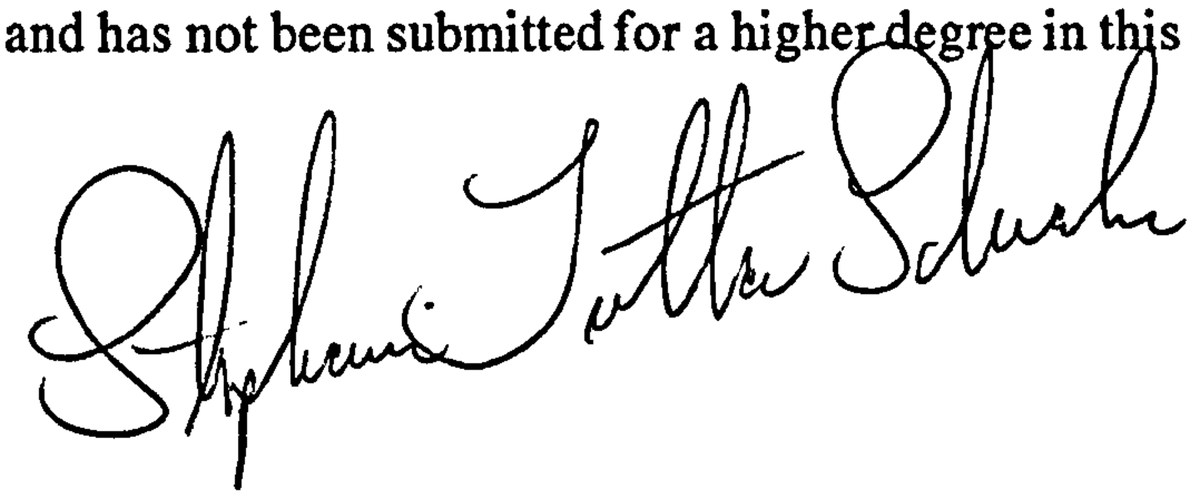
Tom Illiffe I would like to thank for introducing me and teaching me how to use the DataSonde 3 Hydrolab although it was slightly disheartening to know that this instrument could do the water analytical work for this project, which took three years, in 15 minutes; better late than never. For the building of underwater magic tools, I would like to thank Fred Wheeler. Anthony Ball I would like to thank for your tolerance of an ill-tempered, mad scientist in the field. Your help with collecting samples from Stargate Blue Hole till one in the morning was well above and beyond what anyone could ask of a friend, thanks. For humping of heavy and ridiculous amounts of cave diving equipment in hundred degree temperatures to blue hole entrances, I thank Johann Kaiser, Harold and Audun Fosshagen. Sorry about the dog lifting his leg on you Harold.

I would like to also thank UNEXO of Grand Bahama and Robin Kobacker of Grand Bahama Scuba for free air fills and also Robin, thanks for pulling me through the door into the bright lights again, you know what I mean. I also thank the Department of Fisheries for permission to conduct research here in the Bahamas and the Bahamas National Trust for permission to dive in the Lucayan National Parkes Caverns. For financial support, I would also like to thank the GSA grant committee, USA, BCRA, UK, and the University of Bristol for their postgraduate scholarship.

For their unfailing support, I would like to thank my parents, Jutta and Christian Schwabe whom without their encouragement and financial support this dream would never have become a reality. Your countless hours of helping with the rewrites and reading of what now must be pretty boring material helped pull this project together. Can I wipe the foot prints off my back side now? Thank you so much for being a part of this. It makes it all that much more a special chapter in my life. Deine Mausemaedchen hat es endlich geschaft!

Declaration

This thesis is the original work of the candidate except where acknowledgment is given and has not been submitted for a higher degree in this or any other University.



Stephanie Jutta Schwabe

January 1999

Brief Contents

Table of Contents

Figures

Tables

Appendix

1

An Overview of Subsurface Microbiology and Microbial Ecology

General Introduction

It is always amazing that bacteria are regularly discovered in what one would call extreme environments, including those in the subsurface. Extreme because the environment is very cold, very hot, extremely saline, very oligotrophic, or chemically so contaminated that it strains human credulity that life can exist at all in such places. The microbial ecology of many natural environments has been carefully investigated over the years. This section of the thesis will address how microbes live in and alter subsurface environments thought to be extreme even for bacterial life.

The investigation of microbial presence and distribution in subsurface environments, fueled primarily by the need to understand microbial processes in polluted aquifers, has received a great deal of attention over the last decade. As a result of this investigation major discoveries have been made and concepts concerning the subterranean life have been developed. The lessons we were taught years ago in our biology classes stating that life ceases to exist past the first few inches of top soil, is most certainly incorrect in light of more recent data (Wellsbury et al., 1997; Fredrickson and Onstott, 1996). Recent results suggest that there is relatively abundant and resilient microbial presence throughout the earth's crust, extending in some instances several kilometres. (Ghiorse, 1997; Kerr, 1997 and Parkes, et al., 1994). As a result of the more recent discoveries of these deep microbial populations, some of which have been found within basalt deposits some 2.8 km into the earth's subsurface (Stevens, and McKinley, 1995, Furnes, et., al., 1996), and in granites, (Pedersen, and Ekendahl, 1990) many new concepts concerning the subsurface environments and ecology have been brought forth. Some of these new ideas and concepts are: (1) re-thinking of origins and evolution of life (Fredickson and Onstott, 1996), (2) identifying subsurface ecosystems supported by chemosynthetic primary production (Sarbu, et al., 1996) and

independent of surface photosynthesis (Stevens and McKinley, 1995), (3) recognition of the potential of *in situ* bioremediation of polluted groundwater (Chapelle, 1993), (4) role of bacteria in alteration of deeply buried organic matter and potential involvement in fossil fuel formation (Wellsbury et al., 1997), (5) improved methods for oil and mineral recovery (Moses and Springham, 1982), and (6) that the deep biosphere on earth is substantial and may even be greater than the surface biosphere (Gold, 1992).

Along with these recent discoveries, however, it is very apparent there are limitations in one's ability to observe subsurface microbial processes *in situ.* Geochemical and hydrological models (Domenico and Schwartz 1990; Chapelle, 1993) have helped our understanding of geochemical and microbiological processes which combine to effect changes in groundwater chemistry, but it is still not fully understood how microbial communities function *in situ* . Drilling methods are expensive and can introduce many uncertainties into the results and can breach the habitat, exposing the once isolated habitat to other environmental conditions, thereby altering the once pristine site permanently.

Literature indicates (Ghiorse, 1997) that there is a need to assess microbial activity directly in undisturbed subsurface habitats and to collect measurements of real subsurface processes in real time. Ghiorse has suggested that successful methods used in aquatic microbial ecology for *in situ* measurements, especially in the deep sea, be adapted to more near-surface methods for analysis of subsurface environments. Another suggestion is to work with geophysicists who are developing high-resolution, minimally invasive methods to look into the subsurface. However, the best possible way of observing and measuring microbial ecology in the subsurface environment is by actually having a human in place to do so. This can be done by entering the underground via naturally made openings or submerged cave systems (Palmer, 1997) known as "blue holes".

Cave diving has been an on-going practice for several decades (NSS-CDS cave diving manual, 1992; Exley, 1986). Although not recognized in past years as an activity which can be combined with scientific methods, recently a greater understanding of hydrogeological processes through exploration of submerged cave systems has been achieved (Whitaker and Smart, 1997). However, with the advancement of diving techniques, combined with improved water sampling techniques, the amount of information being collected from the subsurface within these cave environments concerning microfauna (Schwabe and Wheeler 1998) and macrofauna (Illiffe, 1992) has increased significantly. This demonstrates that scientific exploration of these environments, using cave diving techniques, can provide the best available *in situ* view of the biology of this particular subsurface environment.

The Bahamas offer a wide selection of sites which would satisfy the criteria needed to do this research project. There are several thousand blue holes scattered

throughout the islands. Cave passages are large enough to access the full extent of the water column, i.e., fresh water to marine water conditions, and earlier research (Whitaker and Smart, 1997) suggested that bacterial activity may be an important part of the water chemistry within cave environments and therefore a vital part of the subsurface ecology.

1:1 Microbes as Key Catalysts of Chemical Processes in the Environment and Subsurface Environments

The geology, hydrology and geochemistry of the subsurface determines to a larger degree the presence, distribution and role of microbes in the subsurface environments as geological agents, i.e. agents of concentration, dispersion and fractionation. Microbes also have a role in reducing and creating porosity in geological substrates via their metabolic by-products (Lovley and Chapelle, 1995; Erhlich, 1990). Until recently, subsurface geochemical processes were thought to be exclusively abiotic and, unfortunately even today with all the evidence supporting a much more extensive microbial role in the subsurface environment, microbial activity and associated metabolic products do not fit easily, if at all, with current groundwater modeling approaches (Lovley and Chapelle, 1995; Shevenell, and Goldstrand, 1997) and therefore are not included in these models.

Subsurface porous and fractured media dictate the movement of groundwater and nutrient flux that may support microbial growth. These conditions may also help to explain, in part, how microbes come to be in the subsurface environment - that is assuming that life began on the surface of the earth and not within the subsurface environment. The source of microorganisms living in the deep subsurface is currently an area of intense investigation. Indirect evidence for microorganisms living on reduced gases emanating from the Earth's core certainly questions whether life actually may have evolved in deep subsurface environments and migrated towards the surface environment (Gold, 1992; Lovley and Chapelle, 1995). Currently, the proposed hypotheses for the presence of microbes in the subsurface are as follows: (1) progeny of the original population buried in sediment or rock during deposition (Balkwill et al., 1994; Chapelle and Lovley, 1990; Fredrickson et al., 1995; (2) microbes were transported into the subsurface via migrating groundwater (Bjornstad et al., 1996; Murphy et al., 1992); and or (3) a combination of (1 and 2). Subsurface microbial populations have been recognized in both the phreatic (Amy et al., 1992; Balkwill, 1989; Balkwill and Ghiorse, 1985; Fredrickson et al., 1995; Kieft et al., 1995; Pedersen and Ekendalhl, 1990; Stevens et al., 1993; West et al., 1992; Whitaker, 1992)

and the vadose zone (Brockman et al., 1992; Fredrickson et al., 1993; Halderman and Amy, 1993; Kieft et al, 1993; Whitaker, et al., 1996).

Microbial activity and the types of reactions which occur *in situ* are very much constrained by the physical environment (Bjornstad et al., 1997). Such physical parameter as porosity and fracture openings will dictate the location of microbes and hence their potential modification of the local environment. Natural openings in rock and the chemical makeup of the rock or sediment also affect the rate and direction of ground-water flow (Martinez et al., 1998) which in turn influences transport and transfer of nutrients into the underground (Fredrickson et al., 1995). The chemistry of the solid media in the subsurface, and the ground-water itself, are important factors influencing the types of microbes found as well as their productivity and distribution. Bacteria are known to preferentially and selectively degrade organic material within sediments and to have a profound effect on the extent to which organic matter is degraded (Figure 1.1) or preserved (Ehrlich, 1990). In the solid phase, i. e., rock and minerals, the secondary alteration products and availability of inorganic electron donors and acceptors will also be factors in the controlling parameters of microbial subsurface distributions.

Aerobic mineralization of organic matter using O_2 as a terminal electron acceptor results in complete degradation to CO_2 and H_2O (Brock, et al., 1996). It is possible for aerobic mineralization of organic material to be completed by a single microorganism, however, in many instances several microorganisms within interacting communities participate in the breakdown of an organic compound, especially polymers (Ehrlich, 1978).

In anaerobic mineralization, the major end products are CH_4, CO_2, H_2, N_2, NH_4^+, S^o, H_2S, Fe^{2+}, Mn^{2+} and PO_4^{3-} (Ehrlich, 1978). Degradation requires NO_3^-, Fe(III), Mn(IV), SO_4^{2-} and CO_2 to serve as terminal electron acceptors (Ehrlich, 1978). Mineralization normally involves a succession of different micro-organisms. Limited availability of electron acceptors or environmental conditions, such as pH, temperature and salinity, will largely determine the extent of mineralization of organic matter within a given environment.

Some organic material is preserved because the conditions are not favorable or a particular organic material is difficult and hence slow to degrade, for example, lignin in wood and kerogen in sediments. Decay-resistant compounds form soil humus (Jackson, 1975). Microbes play a major role in the transformation of organic matter in the soil and sediment in the oceans and fresh water bodies irrespective of the extent of decay. Since biological availability of carbon and other nutritionally vital inorganic elements in the biosphere is limited (Brock, et al., 1996) it is essential that these elements be recycled for the continuation of life. This can only occur if degradation of

dead organic material and waste is efficient which is the major role of microorganisms in the environment.

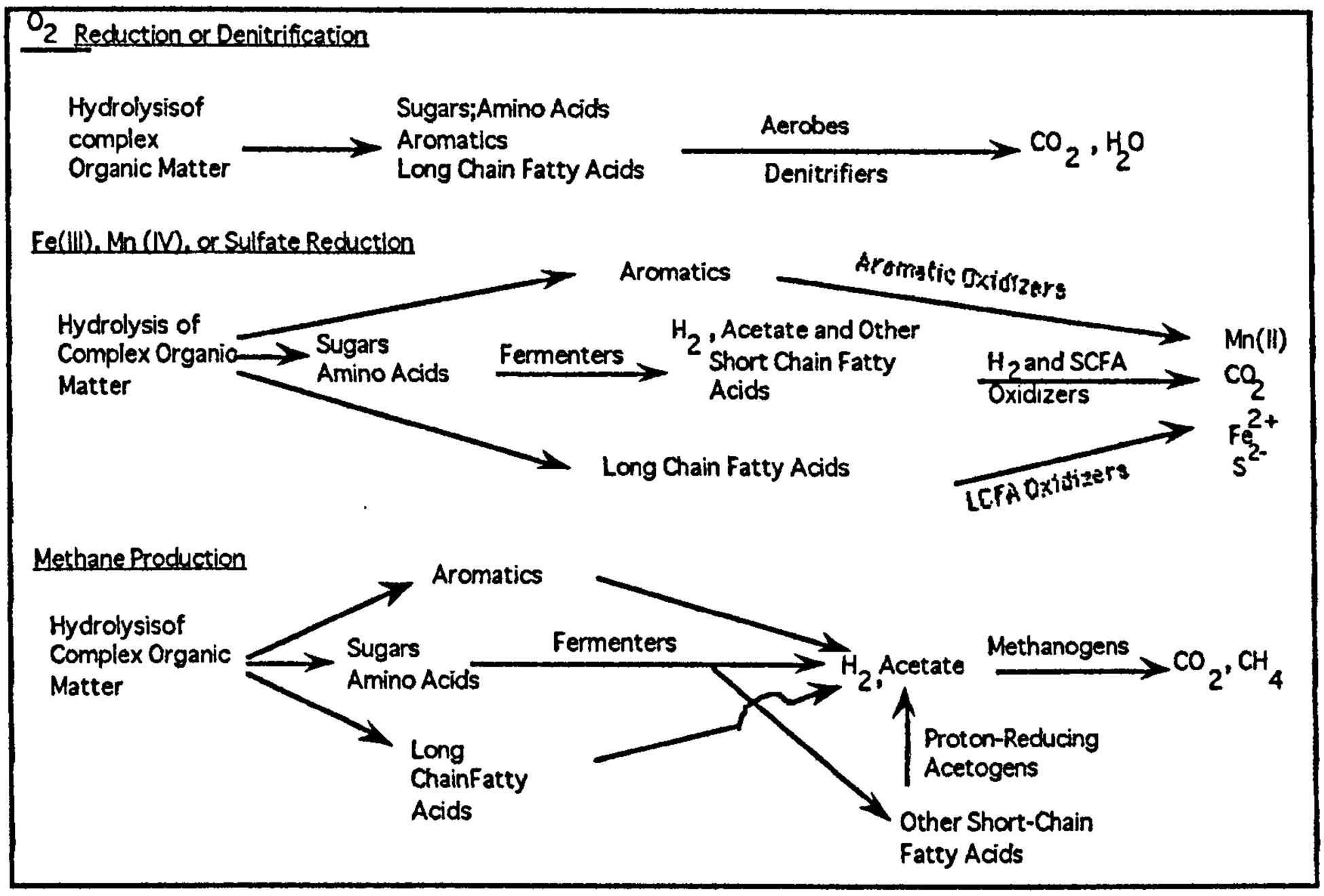


Figure 1.1: Pathway of organic matter decomposition by different microbial processes in sedimentary environments. (Redrawn from Lovley and Chapelle, 1995)

In addition to organic carbon and electron acceptors other parameters which could influence viability and reproduction of microbes in the subsurface are pH, redox conditions, temperature, porosity, toxic compounds, nutrient supply, and probably other conditions of which we are not yet aware. Post-depositional alteration in the subsurface such as diagenesis, including precipitation, dissolution, and recrystallization appear, in many cases, to be microbially mediated processes (Whitaker and Smart, 1997; Lovley and Chapelle, 1995; Ferris et al., 1994; Lovley et al., 1990; McMahon et al., 1991; Monger et al., 1991). Although a great deal of progress has been made in trying to understand subsurface microbial ecology, there is still a great deal to be learned.

Work by Krumholtz and Suflita (1996) and Jorgensen, (1977), has demonstrated that anaerobic bacteria can coexist with aerobic bacteria in locally reducing areas where there are concentrations of organic matter. If the subsurface were a sterile environment, the processes which govern the distribution of chemical species within the ground-water and soils could be determined solely from fundamental principles of mineral equilibria, thermodynamics and chemical kinetics. However, sterile ground-water and soils only exist in extreme conditions of toxic pollution and high temperatures. Hence subsurface processes are both biotic and abiotic, and it is

fundamentally important to understand how the biochemistry of microorganisms, together with the hydrogeochemistry, affects the type and distribution of chemical species in subsurface environments.

Microorganisms demonstrate a remarkable diversity of metabolic processes. Figure 1.2 shows some of the very basic types of energy metabolism. Basically,

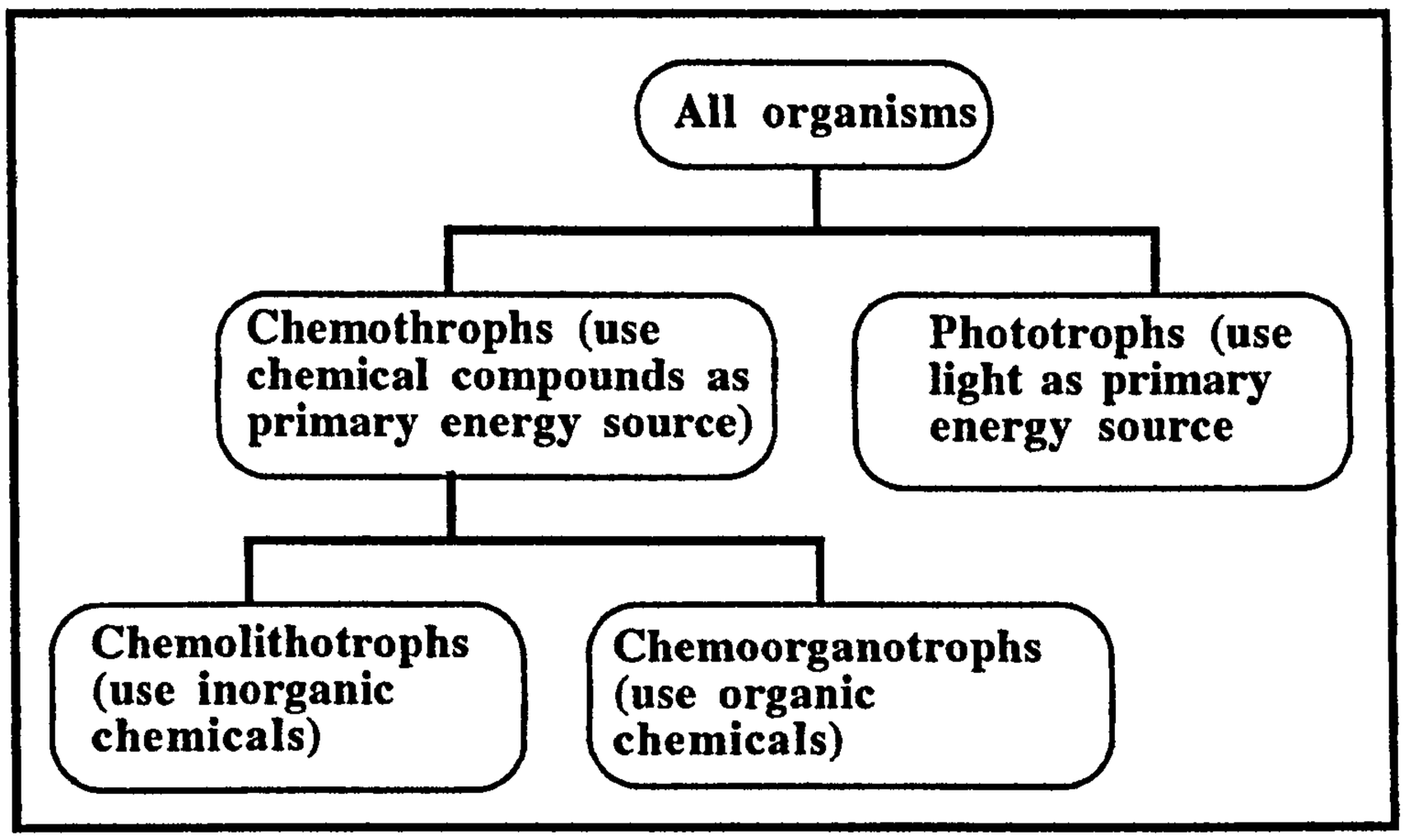


Figure 1.2. Classification of organisms in reference to their energy needs (redrawn from Madigan, 1997)

there are two broad categories, light (phototrophs) and chemical (chemotrophs) (see also Table 1.1). The chemotrophs can be further divided into groups one of which uses inorganic chemicals and the other organic chemicals.

Organisms which can use inorganic chemicals or light as energy sources are in many cases able to grow without any organic compounds, but thrive using carbon dioxide as their sole source of carbon. These organisms are known as autotrophs (meaning, "self-feeding") (Stanier et al., 1986; Madigan et al., 1996; Shively and Barton, 1991). Autotrophs, or primary producers, are of great importance to the biosphere for the main reason that they are able to synthesize organic material from inorganic sources and hence are at the base of most ecosystems. Organisms on the other hand, which require organic material to sustain life, are referred to as heterotrophs (Madigan et al., 1996) and rely on acetotrophs for these compounds.

Table 1.1a Specialist Microbiological Terminology

Term	Definition
Autotroph	Organism able to utilize CO_2 as sole source of carbon
Chemolithotroph	An autotroph that derives energy from the oxidation of reduced inorganic compounds such as H_2, H_2S, Fe^{2+}, or NH_4
Heterotroph	Organism obtaining energy and carbon from organic compounds
Organotroph	Heterotroph

Table 1.1b Specialist Geological and Caving Terminology

Term	Definition
Bioclastic	A sedimentary rock consisting of fragmental or broken remains of organisms, such as a limestone composed of shell fragments, and fecal material.
Cavernicole	Macro-and microfauna living in small openings or pores which do not form part of the main cave passage where cave divers can swim: inhabitants of pores and crevasses.
Choke	Meaning an on-going passage which has been blocked by rocks or some other forms of obstruction: blocked off.
Eolianite	A consolidated sedimentary rock consisting of clastic material deposited by the wind.
Holomictic	Usually refers to a lake that has undergone a complete mixing of its waters during periods of circulation or overturn.
Karst	A type of topography that is formed on limestone, gypsum, and other rocks by dissolution, and that is characterized by sinkholes, caves, and underground drainage.
Ooid	Spherical-subspherical grains, consisting of one or more regular concentric lamellae around a nucleus, usually a carbonate particle, mineral grain or some biological particle, i.e., bacteria, algae etc.
Peloid	Are spherical, ellipsoidal or angular grains, composed of microcrystalline carbonate, but with no internal structure.

Phreatic zone	Water in the zone of saturation.
Ridge	A general term for a long, narrow elevation of the Earth's surface, usually sharp-crested with steep sides, occurring either independently or as a part of a larger mountain or hill. A ridge is generally less than 8 km long (Eardley, 1962, p.6).
Speleothem	Any secondary mineral deposit that is formed in a cave by the action of percolating water: Cave formations.
Vadose zone	Water in a zone of aeration, wandering water.

In the natural environment competition among microorganisms for available resources can be intense. Some microorganisms, however, are able to work together to carry out a particular transformation that neither organism can carry out alone. This type of microbial interaction, known as syntrophy, is critical for the survival of certain groups of bacteria. This form of interaction requires that more than one type of bacteria inhabit the same micro-environment because the product of the metabolism of one organism must be easily accessible to the second. For example, Syntrophomonus species, which use fatty acids or alcohols as energy sources, grow poorly or not at all on their substrates on their own. They require the presence of a hydrogen utilizer, such as a methanogen (Madigan et al., 1996).

1:1:1 Oxygen

Biologically active elements such as oxygen, carbon, hydrogen, nitrogen, iron and sulphur are cycled continuously between oxidized and reduced states in the biosphere (Stanier et al., 1986; Chapelle, 1993). Molecular oxygen, for example, is present in the earth's atmosphere since it is a metabolic waste product of photosynthetic production of reduced organic carbon compounds from carbon dioxide by green chlorophyll-bearing plants and microorganisms (Keeton and Gould, 1986). A balance between oxygen production and oxygen consumption is maintained on a global scale. However, imbalances develop on a local scale, for example localized anoxic areas within the subsurface environment, primarily as a result of the sunlight exclusion and the associated loss of photosynthetic activity.

The flux of oxygen to all sedimentary environments is severely limited by its low solubility in water (Domenico, and Schwartz, 1990). At 20^0 C, approximately 9 mg of oxygen can dissolve in a litre of water (Domenico, and Schwartz, 1990). In rapidly moving waters such as a river aerobic conditions are the norm, but in aquatic sediments or ground-water systems rates of water movement are much slower, and if

slow enough, anoxic conditions may result (Bjornstad, et al., 1997; Whitaker, and Smart, 1996). There are, however, subsurface environments which contain water with oxygen levels in the range of near surface values; 7 to 8 mg/l (Chapelle, 1993). In these instances organic material is very low. From a water quality standpoint, oxygen is one of the more important geochemical components of groundwater. Oxygenated water generally contains low concentrations of undesirable constituents such as dissolved organic carbon, dissolved Fe(II), sulfides, or methane, making such water obviously more suitable for human consumption (White, 1988).

1:1:2 Carbon

The oxygen cycle is very closely intertwined with the carbon cycle. In terrestrial near-surface and marine environments, sunlight is available and CO_2 is reduced to carbohydrates via photosynthesis (Keeton and Gould, 1986). Much of this organic carbon is aerobically re-oxidized via the respiration of animals and microorganisms and returned to the atmosphere and oceans as CO_2. In land caves only a small portion of the CO_2, generated in the soil appears actually to be transported into the karsts' aquifer (White, 1988). Much is assumed to be lost by upward diffusion into the atmosphere although, during the wet season in temperate regions of the world, CO_2 levels can get dangerously high in some dry caves because CO_2 from decomposing humic material diffuses downward, e.g., Mammoth Cave in Kentucky (Palmer A., 1981). This finding resulted in periodic monitoring of CO_2 levels, and in some instances certain sections of the cave had to be closed to the public for a short period of time (Palmer, A., 1981). A similar situation occurs at a cave in Wellington, Australia, known as McCavity Cave, where CO_2 levels get as high as 12%, some of the highest recorded levels in Australian caves (Spencer, 1997). A substantial amount of this CO_2 (Figure 1.3) in marine environments is utilized by calcium carbonate-precipitating marine organisms (Chapelle, 1983; Ehrlich, 1990; Buczynski, and Chafetz, 1991).

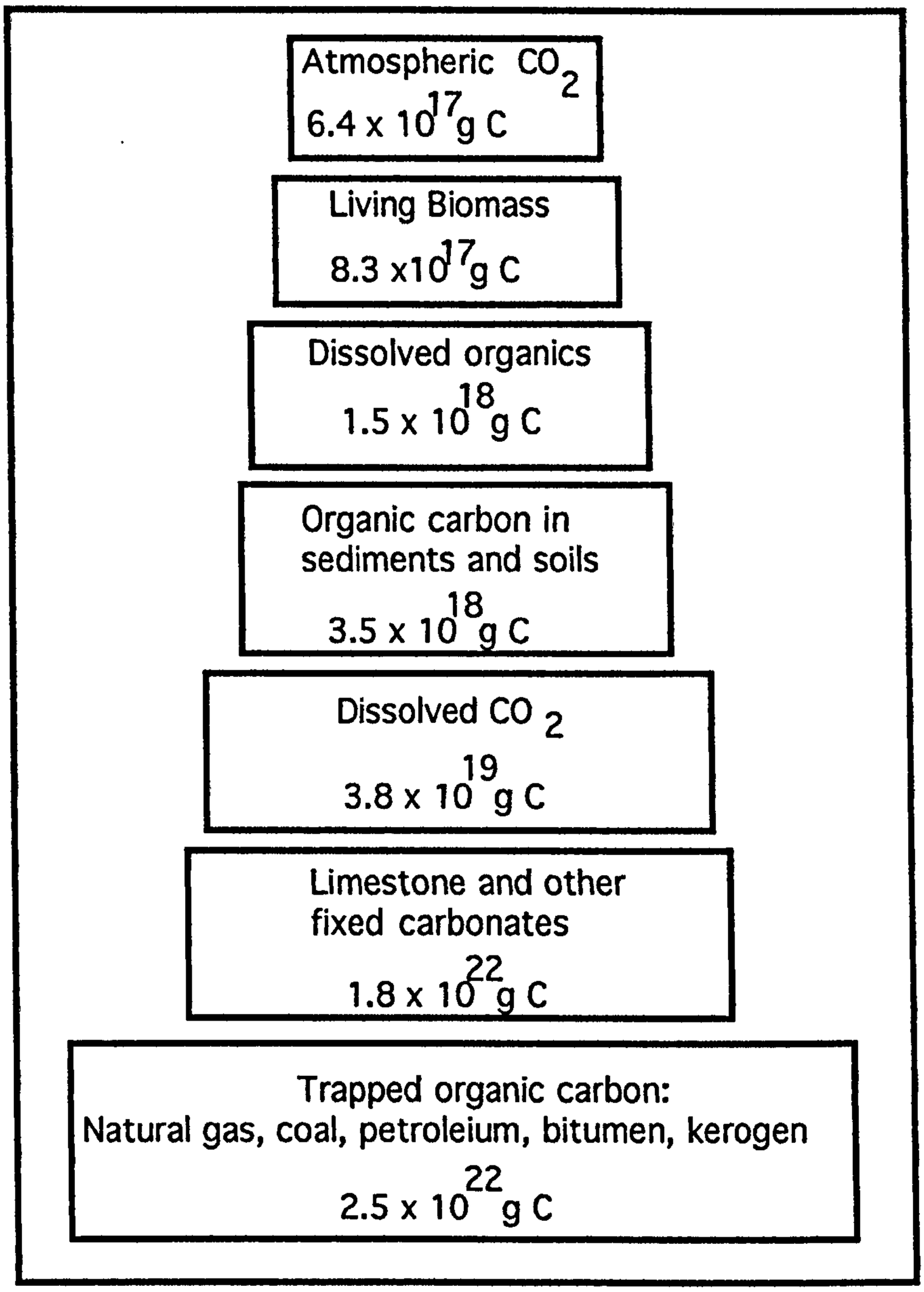


Figure 1.3: Distribution of carbon in the lithosphere of the earth. (redrawn from Ehrlich, 1990)

A large percentage of the organic carbon produced by plant photosynthesis can also be cycled back to CO_2 by anaerobic oxidation (Figure 1.1) once oxygen is removed by aerobic metabolism.

During anaerobic oxidation, fermentative bacteria incompletely oxidize organic carbon, producing organic acids and alcohols (Chapelle, 1993; Madigan et al., 1997; Rueter et al., 1994; Wellsbury et al., 1997). These reduced compounds are then completely oxidized by a sequence of anaerobically respiring bacteria (Figure 1.4) that use inorganic electron acceptors such as Mn (IV), Fe(III), sulphate and CO_2 (Ehrlich, 1990). CO_2 / H_2 and acetate are the major substrates used by methanogenic bacteria to produce methane under anaerobic conditions (Zehnder, 1988). Much of the methane eventually diffuses into oxic environments where oxidation to CO_2 takes place. A

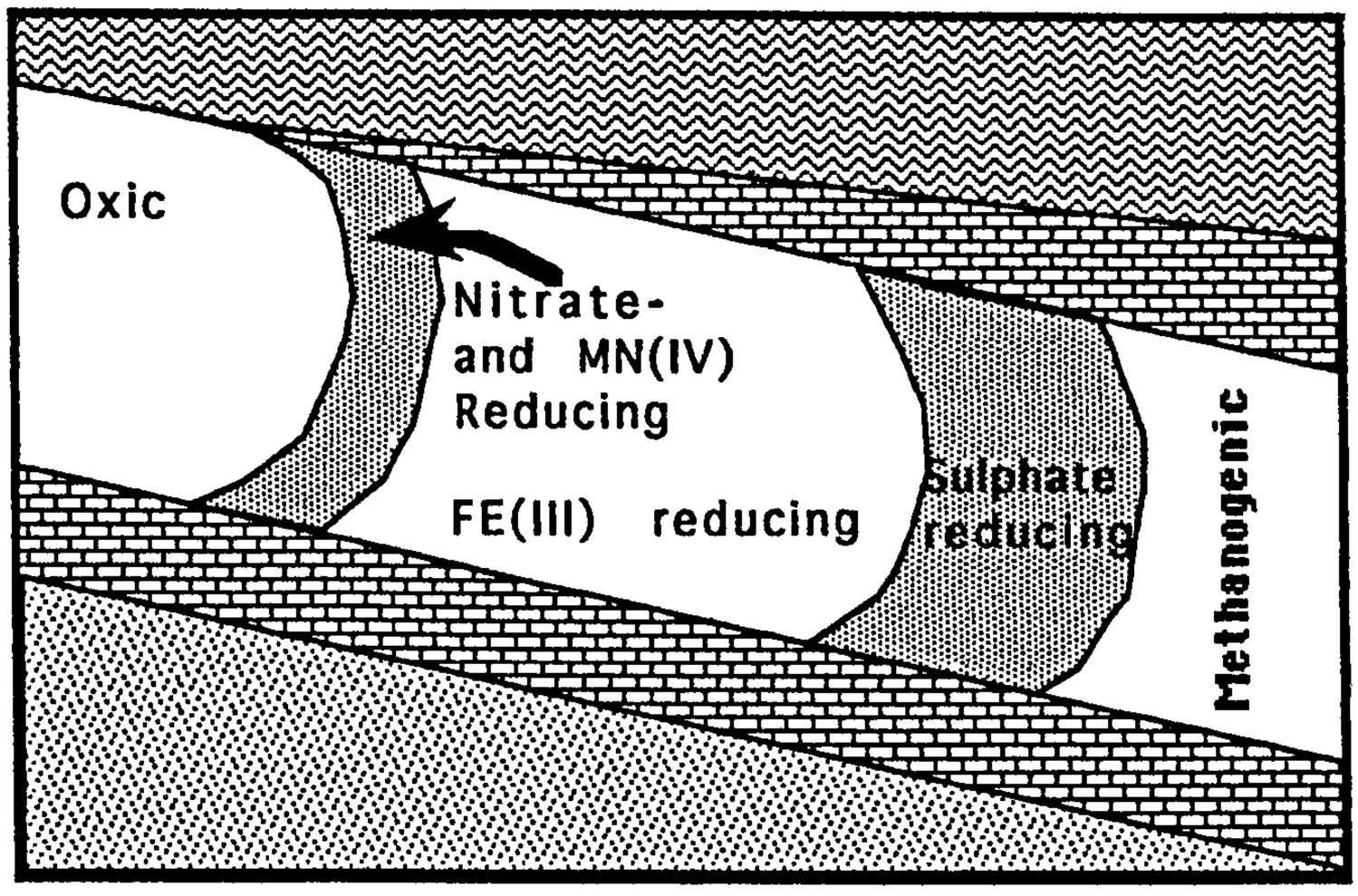


Figure 1.4: Representative distribution of major terminal electron-accepting processes (TEAPs) in deep aquifers (redrawn from Lovely and Chapelle, 1995)

portion of the CH_4, however, becomes trapped in geologic structures and accumulates. CH_4 can also be trapped in gas hydrate deposits. Where aerobic and anaerobic oxidation of organic carbon is incomplete, organic carbon can accumulate over geologic time to form coal, petroleum, and other fossil fuels (Naridon et al., 1995).

1:1:3 Sulphur

The biological transformation of sulphur compounds in natural ecosystems are closely linked to the formation of living biomass and the later decomposition and remineralization of this biomass (Figure 1 5).

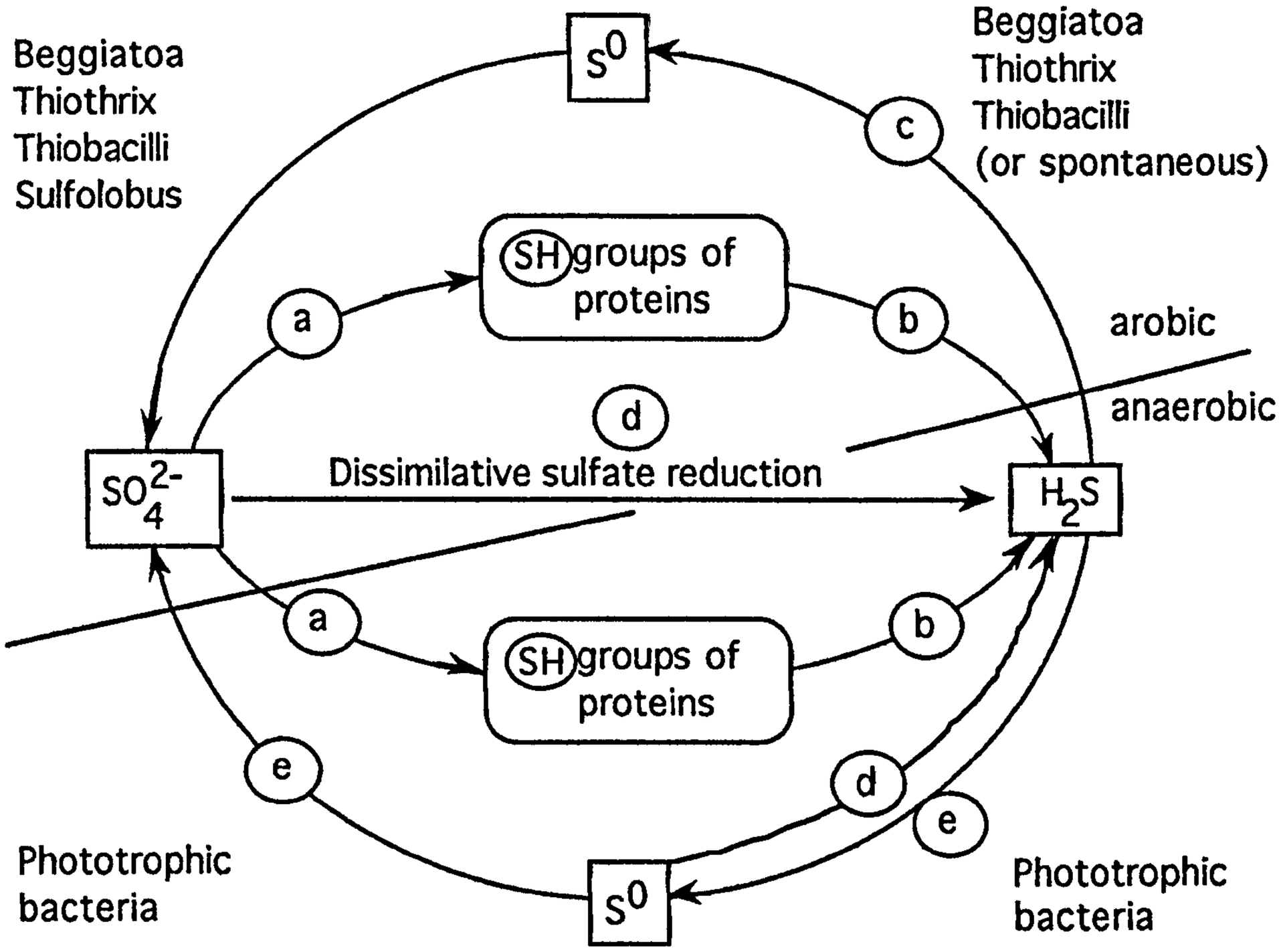


Figure 1 5: Biological Sulphur Cycle-(a) assimilative sulfate reduction can be carried out by a wide variety of organisms, converting SH- to organic sulphur, R-SH; (b) desulphurylation, the opposite of assimilation. On cell death, the reduced SH is released as hydrogen sulfide in both aerobic and anaerobic settings; (c) under aerobic conditions hydrogen sulfide can be oxidized by chemoautotrophs or spontaneously by chemical reaction; (d) SO_4^{2-} and to (b) lesser extent S^0 used in anaerobic respiration for energy generating; (e) oxidation under anaerobic conditions as part of anoxygenic bacterial photosynthesis

The majority of geological sulphur is found in sediments and rocks in the form of sulfate minerals, mostly gypsum, $CaSO_4$, and sulfide minerals such as pyrite FeS_2. (Table 1.2) The largest reservoir of sulphur for the biosphere is found in the oceans in the form of inorganic sulfate (Libes, 1992).

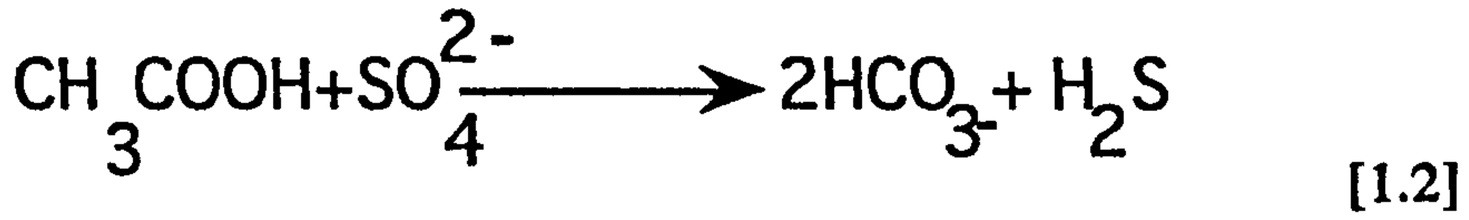
Table 1.2: Sulphur reservoirs and gaseous sulphur emissions on earth

Sulfur reservoirs and gaseous sulfur emissions on earth	
Component	**Reservoir (g)**
I Reservoirs	
Atmosphere	
Hydrogen sulphide	$9.6x10^{11}$
Sulphur dioxide	$6.4x10^{11}$
Sulphate	$16x10^{11}$
Land	
Living organic (mainly plants)	$25,000\text{-}40,000x10^{11}$
Dead organic	$35,000\text{-}60,000x10^{11}$
Inorganic	unknown
Oceans	
Living organic	$0.0000035x10^{11}$
Dead organic	$0.00038x10^{11}$
Inorganic sulphate	$13,760,000,000x10^{11}$
Sediments and Rocks	
Calcium sulphate (Gypsum)	$63,000,000,000x10^{11}$
Metal sulphides (mostly pyrite)	$47,000,000,000x10^{11}$
II Emissions:	
Oceans	$390x10^{11}$
Land	$170x10^{11}$
Salt marshes	$20x10^{11}$

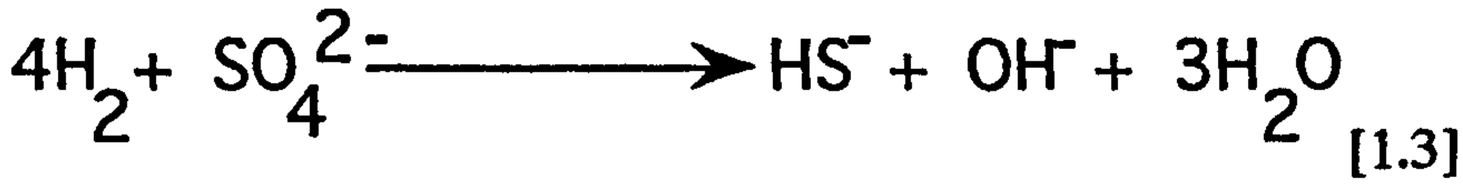
Sulphate reduction is a fundamentally important process in the microbiology of the subsurface environment (Nealson, 1997). Reduced sulphur species such as sulfide, thiosulfate, or polysulfide are produced as a result of organic carbon oxidation in deep sediments. Hydrogen sulfide is produced primarily by the bacterial dissimilatory reduction of sulfate:

$$SO_4^{2-} + 8e^- + 8H^+ \rightleftharpoons H_2S + 2H_2O + 2\,OH^- \quad [1.1]$$

Bacterial sulfate reduction can occur [1.2] heterotrophically or [1.3] autotrophically:

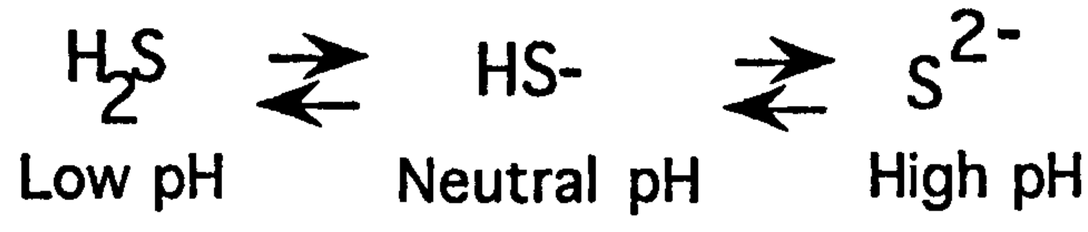


[1.2]



[1.3]

As sulfide diffuses upward, it is oxidized by Fe(III), Mn (IV), and oxygen, reactions which are often microbially mediated. However, the form in which sulfide is present in the environment is very dependent on the pH (Nealson, 1997) of the surrounding media:



[1.4]

At high pH, the dominant form is sulfide; at neutral pH a sulfhydryl group, and at low pH, hydrogen sulfide. Each oxidant produces different sulphur intermediates which can interact with other compounds. Most sulphur oxidizing bacteria are autotrophic, utilizing sulphur compounds as the main source of energy and CO_2 as its main source of carbon (Rosnes et al., 1991). The production of sulfide as a result of sulfate reduction is one of the major biogeochemical differences between fresh water and marine water (Nealson, 1997). In fresh water, sulfate levels can range between 100-250 μM hence S^{2-} production and concentrations are low, whereas in marine waters about 25 mM is normal.

As sulfide reacts chemically (auto oxidation) rapidly with oxygen most bacterial oxidation of sulfide occurs only at the oxic/anoxic boundaries where hydrogen sulfide is defusing up from anoxic areas. Elemental sulphur is relatively stable in the presence of oxygen but is easily oxidized by sulphur-oxidizing bacteria, it is very insoluble in water and the bacteria involved in oxidizing this substrate attach themselves firmly to the cleavage surfaces of the mineral (Madigan et al., 1997). Oxidation of reduced S species results in the formation of sulphate and hydrogen ions, and this lowers the pH of the local environment.

1:1:4 Organic Sulphur

Organic sulphur compounds are found in significant enough quantity within the environment that they also play an important role in the sulphur cycle. The most abundant form of organic sulphur found in the environment is dimethylsulphide (H_3C-S-CH_3). Approximately 45 million tons is produced annually (Madigan, et. al., 1997). Dimethyl sulphide is a degradation byproduct of dimethylsulfonium propionate, an osmoregulatory solute in marine algae (Madigan, et al., 1997). It is also a potential carbon source for microorganisms and is catabolized to dimethylsulphide and acrylate, (a derivative of fatty acid propionate), which is used to support growth (Ehrlich, 1990). Dimethylsulphide, produced in anoxic environments, can be used by microorganisms in methanogenesis, producing (CH_4 and H_2S) as an electron donor for photosynthetic CO_2 fixation in phototrophic purple bacteria [producing dimethyl sulfoxide (DMSO)] which can be used as a terminal electron acceptor by sulphate reducing bacteria (SRB). Although there are other organic sulphur compounds, on a global scale dimethylsulphide production and usage are the most significant.

1:1:5 Iron

Microbial ferric iron reduction is one of the dominant microbially catalyzed redox processes in many subsurface environments (Chapelle, 1993). Only sulphate provides a greater potential for the oxidation of organic matter (Lovley, 1987). One of the major means by which iron is solubilized in nature is by bacterial reduction of ferric iron under acidic conditions (Madigan et al., 1996; Lovley and Chapelle, 1995; Nealson, 1997). Ferric iron is abundant in many microbial habitats but is insoluble and its reduction can be a major form of anaerobic respiration (Widdel et al., 1993). Almost all bacteria need iron nutritionally (Ehrlich, 1990) and special chelating compounds (siderophores) are produced to solubilize the insoluble ferric ion and allow its assimilation. Under anaerobic conditions the soluble reduced ferrous iron can be assimilated. The reduction of ferric to ferrous is an important mechanism for organic matter oxidation but it also presents a major ground water quality problem because as soon as the ferrous-rich water is exposed to oxygen, ferrous iron spontaneously oxidizes to ferric iron, causing the water to taste bad and staining household plumbing fixtures and laundry.

The bacterial aerobic oxidation of ferrous to ferric iron does not yield much energy and, therefore, bacteria need to oxidize large amounts of iron in order to grow. Often ferrous oxidation involves also oxidation of sulphides (e.g. pyrite) and as sulphuric acid is produced this dramatically reduces the pH (1 to 2 pH units). This

situation can present some unpleasant environmental problem where large amounts of ferric iron and other complex ferric salts precipitate out in streams and creek beds; a phenomena known as "yellow boy" by coal miners.

Iron oxidation can also occur at neutral pH. This is most likely to happen in environments where ferrous iron is moving freely between an oxic and anoxic zone (Madigan et al,. 1997). Bacteria such as *Gallionella ferruginea, Sphaerotilus natans,* and *Lepoththrix ochracea* can be found at such interfaces (Madigan et al., 1997). Ferrous iron can also be oxidized in anoxic conditions by certain anoxigenic phototrophic bacteria (Lovley, 1987). The discovery of ferrous oxidizing phototrophs has important implications for understanding the evolution of photosynthesis and for the explanation of the large depositions of ferric iron found in ancient sediments, banded iron formations (BIFs).

1:1:6 Hydrogen

Hydrogen is a common byproduct of microbial fermentations under anaerobic conditions (Chapelle, 1993; Madigan et al., 1997). Hydrogen-oxidizing bacteria can be found at anaerobic and aerobic environment interfaces and tectonically active areas where H_2, associated with hydrothermal degassing, migrates along fractures and faults towards the surface (Chapelle, 1993; Dando et al., 1995). Hydrogen oxidizers are widely distributed in soils and surface waters and there have also been reports of hydrogen oxidizers being found in deep subsurface sediments (Fredrickson et al., 1989). Stevens and McKinley (1995) report what may be an anerobic hydrogen-driven rock-weathering based ecosystem; a system which may be fueled geochemically rather than photosynthetically. Hydrogen production from the interaction between basalt and anaerobic water has been demonstrated in the laboratory and is consistent with the presence of large populations of anaerobic microorganisms which exist entirely as a result of the input of hydrogen gas and is completely independent of photosynthesis (both organic matter and oxygen) (Nealson, 1997)-see, however, Anderson et al., 1998, who questions the validity of the H_2 based communities.

Molecular hydrogen participation in biological reactions is mediated by hydrogenases. Hydrogen, being highly reactive, is generally utilized rapidly by microorganisms and thus prevents accumulation in the environment. It is produced in large quantities only in anaerobic environments. Methanogens are an important group of hydrogen-utilising bacteria, but other bacteria oxidize molecular hydrogen via alternate (Nealson, 1997). H_2 and CO_2 utilizing acetogenes produce acetate and obtain energy from the reaction. Phototrophic purple bacteria can grow as H_2 bacteria under aerobic conditions in the dark (Madigan et al., 1997). Most hydrogen bacteria are facultative

chemolithotrophs (Madigan et al., 1997). When growing autotrophically, aerobic hydrogen utilizing bacteria do the following:

$$6H_2 + 2O_2 + CO_2 \longrightarrow (CH_2O) + 5H_2O \qquad [1.5]$$

Some bacteria, however, are not able to grow on CO_2 exclusively and must have an organic compound as a fixed carbon source. Bacteria which use an inorganic source for energy and an organic source for carbon are known as mixotrophs (Madigan et al., 1997).

1:1:7 Nitrogen

The nitrogen cycle, (Figure 1.6), as other element cycles, is normally balanced but is easily put out of balance on a local scale by agricultural or industrial activities and occasionally by natural causes. When the inbalance occurs, an undesirable amount of intermediates of the cycle accumulate, many of which are toxic (Kuenen, and Robertson, 1988). Nitrogen is essential to all forms of life (e. g. amino acids, proteins, DNA) but only a few highly specialized microorganisms, nitrogen fixers, (e.g. cyanobacteria *Rhizobium, Azotobacter and Azospirillum spp.*) are able to extract nitrogen from the air and turn it into biologically available compounds (Madigan et al., 1997; Kuenen et al., 1988). This process is catalyzed by an enzyme complex known as nitrogenase, a two-component protein containing dinitrogenase and dinitrogenase reductase. Both compounds contain iron, and the dinitrogenase contains molybdenum as well. The iron and the molybdenum are contained in a cofactor (FeMo-co)

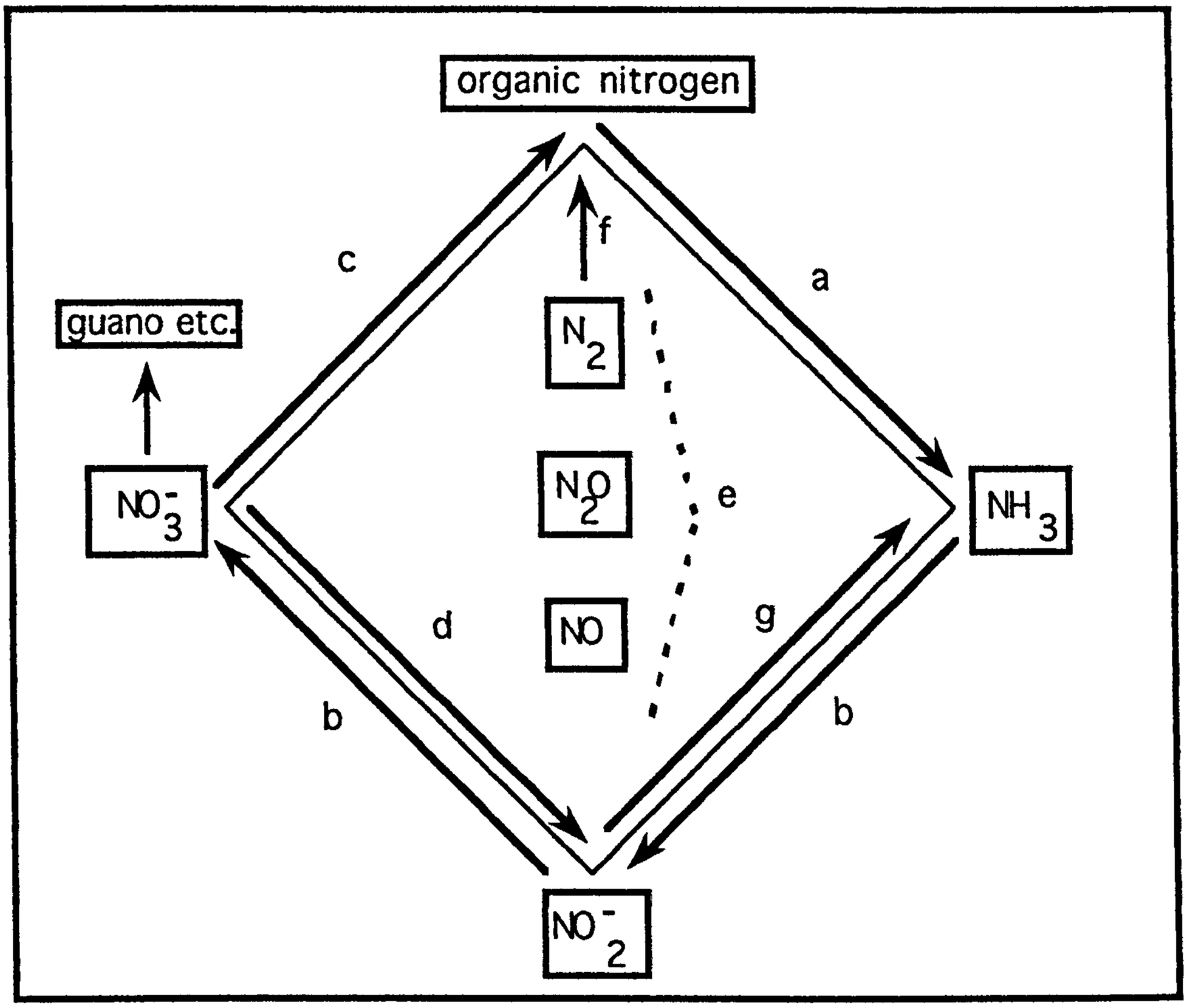


Figure 1.6: The nitrogen cycle. (A) ammonification (aerobic and anaerobic), (b) autotrophic nitrification (strictly aerobic), (c) nitrate assimilation (aerobic or anaerobic), (d) nitrate reduction (generally anaerobic), (d+e) denitrification (generally anaerobic), (f) nitrogen fixation (both aerobic and anaerobic), (d+g) nitrate ammonification. (Redrawn from Ehrlich, 1990)

and it is at this iron-molybdenum site where N_2 reduction takes place. Nitrogen fixation is very reductive in nature and the process is inhibited by oxygen because dinitrogenase and dinitrogenase reductase are rapidly and irreversibly inhibited by the presence of oxygen. The organisms which fix nitrogen have their nitrogen-fixing sites protected by oxygen inactivators. This process can occur either by rapid removal of oxygen via respiration, production of oxygen-retarding slime layers, or by compartmentalization of the nitrogenase enzyme in what is generally known as heterocysts. It is also possible for an organism to inactivate oxygen by complexation with specific proteins (Madigan, et al., 1997).

The decomposition of plant and animal remains in anaerobic conditions can result in the production of reduced nitrogen compounds like ammonia (NH_3) and ammonium (NH_4^+). Nitrifying bacteria use inorganic nitrogen compounds like (NH_3) and nitrite (NO_2^-) as electron donors under aerobic conditions (Madigan et al., 1997) and can be found widely distributed in soil and water (Chapelle, 1992). Fredrickson et

al. (1989), showed that nitrifying bacteria are also present in deep subsurface sediments. Approximately 85% of nitrogen fixation on earth is of biological origin of which 60% occurs on land and 40% occurs in the oceans (Chapelle, 1993).

1:2 Deep Subsurface, Marine Sediment and Cave Microbiology

The deep subsurface, which can extend for several hundred kilometres below the soil surface, was thought for a long period of time to be an abiotic wasteland. However, due to extensive drilling programs, e.g. The International Ocean Drilling Program, and investigation of oil reservoirs (Stetter et al, 1993), and deep aquifer (Fredrickson and Onstott, 1996) information to the contrary has been gathered. In samples collected aseptically (Parkes et al, 1994; Madigan, et al., 1997; Wellsbury, et al., 1997) from bore holes ranging in depths up to 2.8 km a variety of viable bacteria have been found. Discovery of the extended biosphere brought about worries concerning the safety of water in deep aquifers and led to questioning the safety of burying toxic waste since more than half the world's population depends on aquifers for drinking water. Pedersen and Ekendahl's (1990) results showed that bacterial counts from water samples collected below 1000 metres had total microbial counts as high as 10^4 and 10^6 cells/ml. In the North Sea in oil reservoirs 1.2 to 6 km below the sea floor, in temperatures ranging from 60 to 200°C, 1 x 10^5/ml cells were found in samples where in other samples from similar depth the number of cells ranged from 1 x 10^4 to 5 x 10^5/ml (Nilsen et al., 1996). Microorganisms at these extreme depths, could very well be able to alter the solutes in the either natural or anthropogenic (e.g. secondary oil recovery).

Microorganisms have been found not only in deep sediments but also in deep igneous deposits such as granite (Pedersen, et al., 1997) and basalt (Stevens, and McKinley, 1995) which are virtually void of other organic material. Marine sediment cores from 750 metres have been found to have surprisingly high bacterial populations 1.8 x 10^6 cells / ml (Wellsbury et al., 1997).

Although the metabolic rates of deeply located bacteria are usually low by comparison (Chapelle and Lovley, 1990) with surface microbial activity, the significance of these results is that over long periods of time microbial processes could be responsible for geochemical reactions in the deep subsurface previously thought to be abiotic. Metabolic interdependence may explain the ability of some bacteria communities to survive in subsurface oligotrophic environments. Another factor which seems to limit the extent of life into the earth's subsurface is temperature. Bacteria can grow at least to temperatures up to 113°C and survive short exposures to even higher temperatures (Stetter et al., 1993) which covers the top 4 kilometres or so of the earth's

subsurface environment (using a thermal gradient of the Earth's surface of 30°C / km (Pain, 1998).

Water-filled cave environments have essentially constant temperatures near the annual mean of the region, and away from entrances are devoid of light (Whitaker and Smart., 1997; Vacher and Bengtsston, 1990;, Smart et al., 1988; Palmer A.N., 1981). Photosynthetic production based on the latter conditions is not likely and therefore a large percentage of cave communities depend on allochthonous input of organic material from the surface as the base of the food chain (Kane et al., 1994). This may well be the case for caves found in Romania (Kane et al., 1994) but not for caves found in the Bahamas (Whitaker and Smart., 1997). In the Bahamas, organic material comes in from the surface as well as with tidal waters which flush the cave systems twice a day (Whitaker and Smart., 1997).

Caves in Dobrogea, southern Romania, have been found to contain chemoautotrophic bacteria, evidence based on experimental results using radio-labeled bicarbonate incorporation by microbial mat populations. It is speculated that bacteria are deriving their energy from the oxidation of H_2S from magmatic origin and thus this system may have characteristics in common with the unusual communities from near deep-sea thermal vents (Kane et al., 1994).

Microorganisms have also been detected in caves in New South Wales Australia (James, 1994). Many of the microorganisms that are found in deep caves (according to James) are identical to those found on the surface. James suggests that they are transported into and around caves by water, air sediment and animals. By far the most significant effect of microorganisms in deep caves is the solution of limestones resulting from a net production of carbon dioxide by the degradation of organic materials. The activities of microorganisms can be monitored through studies of carbon dioxide and other gases in the cave atmosphere and by changes in water chemistry, especially those associated with the carbon cycle. James further proposes that many of the microorganisms found in deep caves are opportunistic, becoming active only if they find favorable conditions for their growth and finally, in the cave depths it is not possible, according to James, to separate biogenic from inorganic processes.

According to James, microorganisms through their life processes (oxidation-reduction reactions) are consumers and producers of acids and bases (e.g. organic acids, CO_2, OH^-, HCO_3^-, NH_4^+ and H^+). Therefore they have a significant effect on the pH and alkalinity of cave waters, resulting in shifts in the carbonate equilibrium. James also suggests that in deep cave environments microbial acid production far outweighs its consumption, making microbial solution of limestone common and microbial precipitation of calcite rare.

Bacteria have also been studied from caves in the Nullarbor region (James and Rogers, 1994). The Nullarbor region is a vast area next to the Great Australian Bight

on the southern Australia coast. It comprises the world's largest karst area and by far the largest arid karst with an area in excess of 200,000 km^2 (James and Rogers, 1994). Within the Eocene and Miocene aged limestone (James and Rogers, 1994) is one of the longest caves and largest chambers in Australia. Within the enormous flooded passages divers have reported curtains of gelatinous substance attached to rock surfaces which once examined under an optical microscope was seen to contain filamentous bacterium with the gelatinous substance containing concentric circles of calcite crystals (James and Rogers, 1994).

The water within this cave system is dominated by sodium chloride. The water columns in the caves are at times stratified; after intense rainfall a lens of "fresh" water may be found on top of the brackish / saline interface. The water itself is clean, mostly oxic, not polluted and saturated with respect to calcite (James and Rogers, 1994). Water left within sealed vials became anoxic and H_2S could be detected by smell. Water samples collected from the interface of the "fresh" and the brackish waters contained H_2S and were anoxic. From these results, James and Rogers concluded that sulfate reducing bacteria are present throughout the Nullarbour Aquifer.

1:2:1 Microbial Interaction with Chemical Gradients

The vertical zonation of electron donors and electron acceptors in chemically stratified marine water columns resemble the distribution observed in marine sediments (Ramsing et al., 1996; Sorokin, 1970). The main difference, according to Ramsing et al.,1996, is the spatial scale involved; the scales between sediment and water column is at least 3 orders of magnitude greater. Principal changes in basic metabolism that occur over a few millimetres in coastal sediments are extended to several metres in stratified water columns (Ramsing et al., 1996; Lee, 1992; Sorokin, 1970; Repeta et al., 1989). The bacterial populations and the coinciding chemical environments of many permanently stratified marine water bodies have been investigated by traditional cultivation-dependent methods (e.g.., the Black Sea, Cariaco Trench and more) (Ramsing et al., 1996).

A complementary investigation to Ramsing et al., (1996) of the Mariager Fjord was done by Teske et al., (1996). Distribution of sulfate-reducing bacterial populations of the stratified marine water column was investigated by molecular and culture-dependent approaches in parallel. Their result indicated specific bacterial populations in different water column layers and revealed a highly differentiated pattern of rRNA- and rDNA-derived PCR amplificates (e.g., PCR-amplified 16S rRNA and DNA encoding rRNA (rDNA), suggesting active and resting bacterial populations within the water column. Their results further suggest the increased presence and activity (by at least 1

order of magnitude) of sulfate-reducing bacteria within and below the chemocline.

Bacterial counts were used also and they found a similar distribution of cultivatable sulfate-reducing bacteria in the water column of Mariager Fjord, approximately 25 cells and 250 cells per ml above and below the chemocline, respectively. Teske et al., (1996) found that *Desulfovibrio*- and *Desulfobulbus*- related strains occurred in the oxic zone. Ramsing's findings were that long slim rod-shaped bacterium was found in large numbers in the oxic part of the chemocline, whereas large ellipsoid cells dominated at greater depth.

Jørgensen (1982) describes three main types of O_2-H_2S interface environments; chemoclines of stratified water bodies (e.g. Black Sea, Cariaco Trench and Solar Lake, a meromictic lake), shallow marine and fresh water sediments in association with sites of accumulation of organic matter (e.g. bacterial mats) and hydrothermal vents. Three examples of sites of O_2-H_2S interfaces that Jørgensen describes in his 1982 work are, the Black Sea, Solar Lake and a Beggiatoa mat. The dimensions and rates of the three sites are given in Table 1.3. The physical differences between the three sites gives an interesting insight as to the viability of the environments in which these interfaces can be found.

Table 1.3: Comparison of three O_2-H_2S interfaces: Dimensions and Rates (reproduced from Jørgensen, 1982)

	Black Sea	**Solar Lake**	***Beggiatoa* mat**
-temperature /°C	6	50	20
-O_2-H_2S interface	35cm	10 cm	50μm
-depth (proportion	700000	2000	1)
-H_2S residence time	5 days	10-20 min	0.6 s
-(proportion bacterial H_2S	700000	1500	1)
-oxidation (%) concentration at	0	30-50	100
-CO_2=C H_2S /μm	1-3	2-4	3-5
H_2S oxidation rate			
-peak / (μmol l^{-1} d^{-1})	0.8	250	250000
-area / (mmol m^{-2} d^{-1})	10	20-30	12

Studies concerning chemically stratified water (Jørgensen 1982), were found not to have sufficient depth resolution, based on sampling problems, to accurately

identify the coexisting concentrations of O_2 and H_2S. Jørgensen found that the interval of O_2-H_2S coexistence is generally very narrow compared to the total depth of the interface below the water surface and, as a result, is difficult to detect. One of the major problems with sampling open bodies of water is wave motion which causes samplers to move vertically, making accurate depth sampling sometimes impossible. Collecting samples from the stratified layer was also made difficult as a result of the samplers moving between the layers of the chemocline and mixing them.

Within the 2000 m deep water column of the Black Sea, the anoxic layer begins at 150-200 m depth and the O_2-H_2S interface within this body of water is 35 m thick (Jørgensen, 1982). Comparatively, Solar Lake has a maximum depth of 5 m and a temperature of 50°C compared to the average temperature of the Black Sea of 6°C (Jørgensen, 1982). In Solar Lake however, the O_2-H_2S is found at 3.6 m below the water surface and is only 10 cm thick. Jørgensen found that the exact position of the interface varied between day and night due to photosynthetic activity of planktonic cyanobacteria which oxidized the sulphide and produced oxygen in the light. Jørgensen also found that high rates of CO_2 fixation occurred at night within the chemocline and above it when thiosulphate was present in the water strata.

The dense Beggiatoa mat examined by Jørgensen formed a thick white sheet 0.5 mm thick on the surface of the black mud in which intensive sulphate reduction took place. Oxygen and sulphide showed very steep gradients above and below the mat surface, explaining a potentially high rate of H_2S oxidation compared to the Black Sea chemocline. An important difference, Jørgensen notes, between the large-scale and small-scale chemoclines are the intermediate product of sulphide oxidation. He states that in the Black Sea, the Cariaco Trench, the Solar Lake and several other stratified water bodies, sulphate and thiosulphate are the immediate products of sulphide oxidation, and thiosulphate has a concentration maximum in the chemocline. In bacterial mat environments, elemental S° is an important intermediate. The advantage for the bacteria in producing S° is that bacteria can keep it either intracellularly or it can precipitate it largely as non-diffusible granules around the cells. The S° is therefore available for further oxidation and reduction whereas if $S_2O_3^{2-}$ is produced, it can diffuse away from the interface within a few seconds and the energy source would be lost to the bacteria.

Chemocline in the water column can also be created by bacteria (Jørgensen, 1982; Fenchel and Glud, 1998). Bacteria may form fragile slime webs or veils which are suspended over decaying animals and plants. The veil can sharply separate the overflowing oxic body of water from the anoxic, sulphidic water. The veil formation seems to serve two main purposes (Jørgensen 1982; Fenchel and Glud, 1998). One reason is to position cells exactly at the narrow O_2-H_2S interface and the other is to create and stabilize the interface, maximizing H_2S availability for the organisms.

1:3 Hydrothermal Vents and Hot Springs

Submarine hydrothermal vents, which are usually found at mid-oceanic spreading centres, are in depths of water ranging from 2000 to 3000 metres (Deangelis, et al., 1991). Two uniquely different types of vents (Figure 1.7) exist at the ocean floor spreading centre; warm vents ejecting hydrothermal fluid at temperatures of 6-23°C (into surrounding sea-water of 2°C) and hot vents, (270-380°C) commonly referred to as "black smokers" (Jannasch, and Mottl, 1985). Flow rates vary between these two vent types; warm vents discharge fluids at about 0.5-2 cm /sec, whereas hot vents discharge fluids at approximately 1-2 m /sec (Jannasch, and Mottl, 1985).

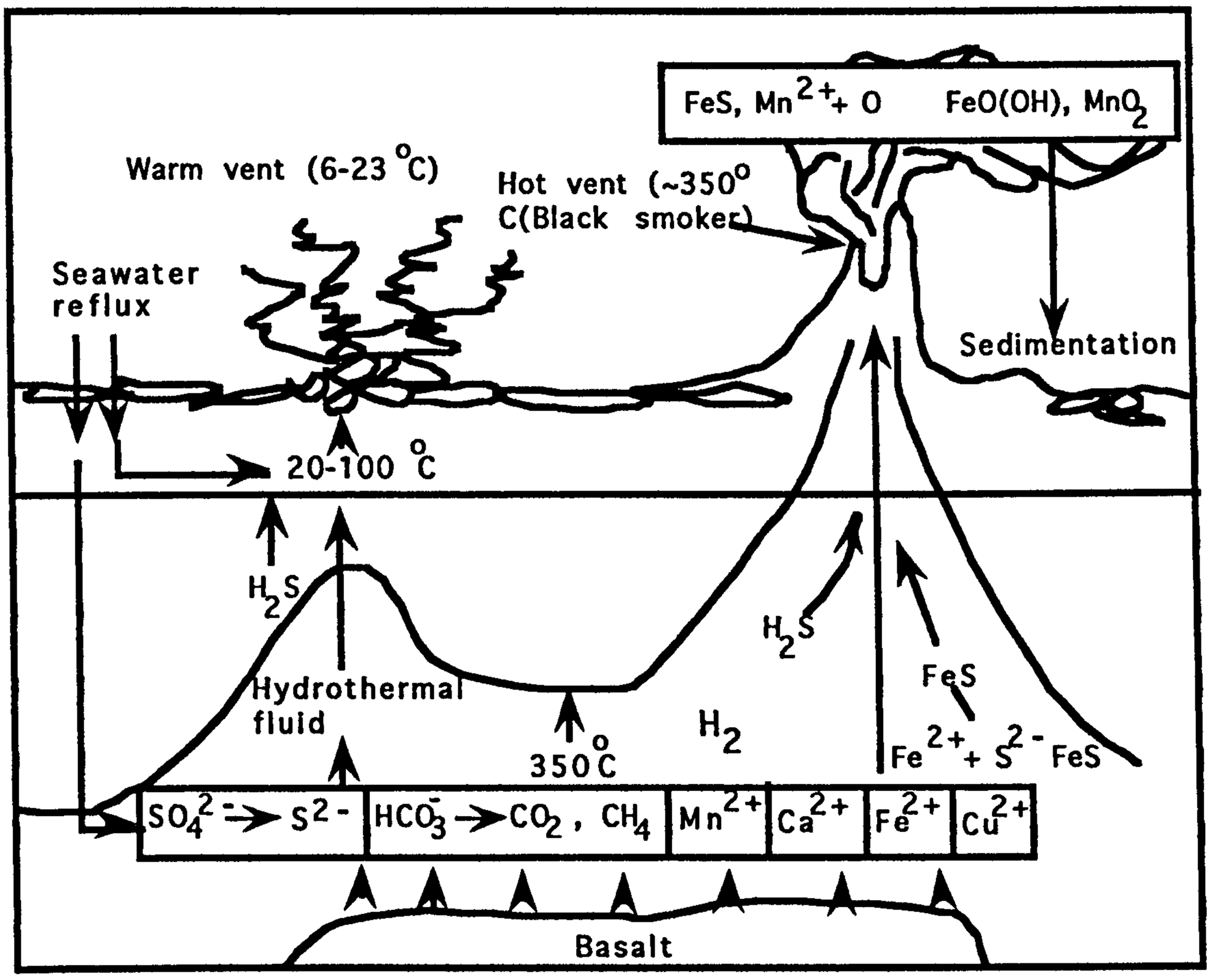


Figure 1.7: Schematic diagram of the hydrothermal vents and hot springs which can be found at the mid-Atlantic ridge and other tectonically active places.

Warm vents, however are not exclusively a deep water phenomena. Shallow water hydrothermal vents have been described from White Point, California (Stein, 1984; Jacq, et al., 1987; Trager, and Deniro, 1990) Baja California (Vidal, et al., 1978) New Britain (Ferguson, and Lambert, 1972), the Bay of Plenty, New Zealand (Sorokin, 1991; Sarano, et al., 1989; Kamenev, et al., 1993), the Kuril Islands (Tarasov, et al., 1990) southern Japan (Hashimoto, et al. 1993; Naganuma, 1991) and

off Iceland at Kolbeinsey, to the north (Fricke, et al., 1989) and to the south at Steinaholl, south of Reykjanes Peninsular (Olafsson, et al., 1991) and Milos, Greece (Dando, et al., 1995). These vents produce either warm fluids (7-27°C) or gases composed of 71-92% CO_2, with approximately 92.0% CH_4, 5.0% H_2S and 3.0% H_2 (Dando, et al., 1995).

Chemical analyses of hydrothermal fluids from warm vents demonstrate the presence of large amounts of reduced inorganic compounds (Karl et al., 1980) and some organic carbon compounds, some of which are H_2S, Mn^{2+}, H_2, CO, CH_4 and from some vents, NH_4^+. Spreading centre hydrothermal warm vents are of particular interest because of elevated microbial activity relative to ambient deep-sea water (Fliermans et. al., 1993). In and around the vents, large populations of sulphur-oxidizing chemolithotrophs such as *Thiobacillus*, *Thiomicrospira*, *Thiothrix*, and *Beggiatoa*, have been found. Other vents yield nitrifying bacteria, hydrogen-oxidizing bacteria, iron- and manganese-oxidizing bacteria, and methylotrophic bacteria which are probably living on CO and methane emitted from the vents (Cavanaugh, 1985).

Black smokers, the second major type of vent found at spreading centres, emit superheated hydrothermal fluids (270-380°C) (Jannasch, and Mottl, 1985). This superheated fluid does not boil because of the elevated ambient hydrostatic pressures. At a depth of 2,600 m water does not boil until it reaches temperatures of about 450°C. What does happen, however, is that super-heated fluid comes in contact with super-cool sea water, causing dissolved minerals within the fluid to precipitate out (Madigan et al., 1996). Minerals such as metal sulfides and zinc-rich precipitates are responsible for forming the classic towers referred to as "chimneys.

The most likely group of bacteria which can live near these super-hot fluids are a mixture of both aerobes and anaerobes (Tuttle, et al., 1983). Although no known bacteria live in the hot (250°C) fluids, prokaryotes have been collected from 140°C fluids (Tuttle, et al., 1983) but can actually grown in a lab at temperatures of 113° C (Blöchl, et al., 1997). The chemolithotrophic bacteria fix CO_2 autotrophically into organic carbon by using enzymes of the Calvin cycle, some of this carbon being used by symbiotic association of bacteria and animals. The primary producers at the deep sea sites are chemolithothrophic rather than phototrophic, and bacterial rather than eukaryotic.

Prior to the discovery of these vents, it was thought that life was not possible at these depths, due to the extreme pressures, cold temperature (2-3 °C), lack of light and lack of particulate organic matter (Libes, 1992). Finding this chemosynthic community surrounding the spreading centre vents has stimulated new views on when and how life

may have begun on this planet and the extreme conditions in which life forms can survive.

1:4 Precipitation and Dissolution of Minerals by Micro-organisms

A prerequisite for mineral precipitation is that the solution be supersaturated by the mineral to be precipitated (Fortin et al., 1997). Over-saturation is symptomatic of an activation energy barrier that inhibits the spontaneous formation of insoluble precipitates from solution (Stumm, 1992). Nucleation is the point at which the activation energy barrier is breached and involves the growth of molecular clusters (i.e. critical nuclei) (Fontin et al., 1997). Nucleation can occur either homogeneously or heterogeneously (Stumm, 1992). Homogeneously, the stable nuclei develop through random collisions between ions in an over-saturated solution. Heterogeneous nucleation involves development of nuclei on surfaces of foreign solids (Fortin et al., 1997). Basically, heteronucleus is a scaffold or template of similar atomic spacing that encourages mineral precipitation (Fortin et al., 1997; Thompson et al., 1997).

Bacterial involvement in mineral precipitation involves catalytic reactions in aqueous environments (Thompson et al., 1997) and indirectly geochemically reactive solids (Fein et al, 1997). Bacterial metabolic activity can influence the surrounding environment and can trigger changes in solution chemistry that lead to over-saturation . This can cause mineral precipitation by lowering the activation energy barrier for nucleation reactions. However, bacterial cell surfaces can generate heterogeneous nucleation and precipitation which can form on the inside, outside, and in some instances some distance from the cell (Stumm., 1992).

1:4:1 Iron

Iron is the 4th most abundant element in the Earth's crust (ca 5%) and oxidation and reduction is strongly microbial mediated, both directly and indirectly. Large-banded iron formations (BIF), a major economic source of iron, occurred around 2.8 - 1.6 billion years ago (Frazier, and Schwimmer, 1987). BIFs' are characterized by alternating layers between high silicate and low ferrous iron, to low silicate and high ferric iron (Andel, 1994). Chert was deposited in BIF because no silicate-depositing microorganism (e.g., diatoms, radiolarians) had yet evolved. As oxygen was generated by photosynthesis, ferrous iron was oxidized and precipitated. Gradually ferrous iron reserves became largely depleted, allowing the oxygen content of the atmosphere to increase to levels associated with an oxidizing atmosphere. Previously iron weathered

from volanic rock was in the reduced form ferrous, which accumulated in large amounts along with pyrite and manganese in the sea (Fenchel, and Blackburn, 1979). With the onset of oxygenic photosynthesis by cyanobacteria, sulphide was oxidized to sulphate and ferrous iron to ferric iron (Shively, and Barton, 1991). Ferric iron precipitation occurred over wide areas where the ferrous-containing deep waters came into contact with the oxygenated overlying waters. Oxygen could only accumulate in the atmosphere when the sulphur and iron oxidation in the oceans was completed about 1.6 billion years ago (Frazier, and Schwimmer, 1987).

Micro-organisms are also implicated in the mobilization of iron from granite and its re-precipitation (Lundgren, and Wilver, 1980). The reduced sulphur components of pyrite and mercasite under oxic conditions (Figure 1.8) are oxidized to sulphuric acid by *T. ferrooxidans* and *Thiobacillos thiooxidans* in an initiator reaction. pH decreases, and the iron is dissolved as ferrous ion and is then oxidized by *T. ferrooxidans* to ferric ion: upon neutralization of the water, the ferric ion is precipitated out as $Fe(OH)_3$. At low pH (< 3.5) ferric ion is stable and oxidises further to pyrite in the propagation reaction providing more ferrous for *T. ferrooxidans*, a community of acidophilic bacteria which can increase the rate of pyrite weathering by a factor of 10^5 (Lundgren, and Wilver, 1980).

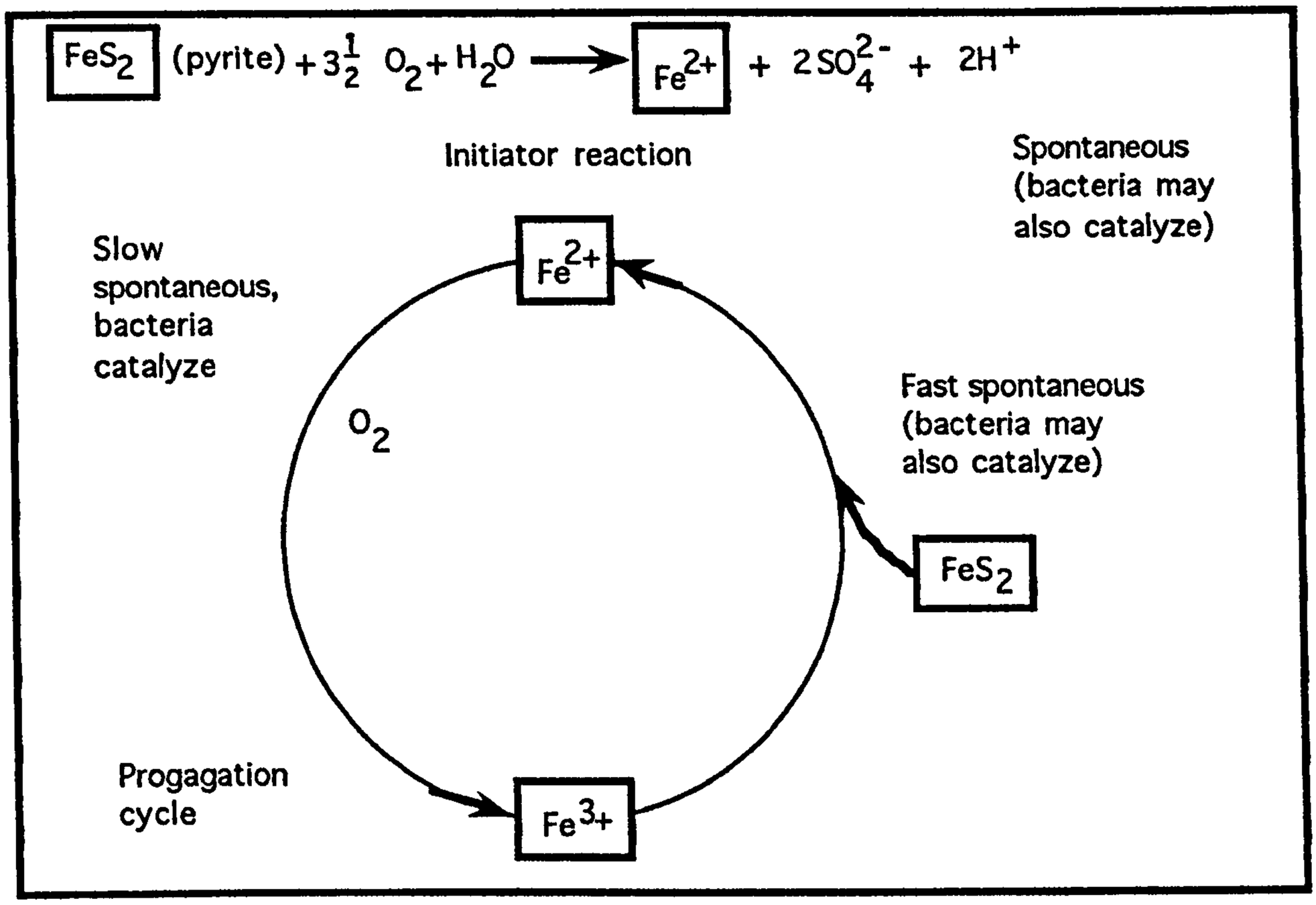


Figure 1.8: Role of iron-oxidizing bacteria in oxidation of pyrite (redrawn from Madigan et al., 1997)

Deposits of pure iron are most likely a result of microbial leaching processes occurring over millions of years (Ludgren, and Wilver, 1980). Solubilization of iron may also occur by organic acids like humic acids, for example (Watkinson, 1978). The oxidation of ferrous to ferric iron can also be carried out at neutral pH by other bacteria, the iron bacteria *Gallionella* and *Siderocapsa*, for example (Gibson, and Parkinson, 1984). The end product of these conversions are bog iron ore and marsh or swamp ore (Giblin, 1988).

Iron plays an important role in cellular respiration, being a key component of the cytochromes and iron-sulphur proteins involved in electron transport (Ehrlich, 1990). However, for a cell to incorporate iron it must be in solution which presents a problem because most inorganic iron salts are highly insoluble. Many organisms produce specific iron-binding agents called siderophores, which solubilize iron salts and transport iron into the cell (Madigan et al., 1997). One major group of siderophores consists of derivatives of hydroxamate acid, which chelate ferric iron very strongly. As soon as the iron-hydroxamate has passed into the cell, the iron is released and the hydroxamate leaves the cell to be used again for iron transport (Madigan, et al., 1997).

1:4:2 Calcium

Calcium is the most abundant of the alkaline earth metals (Nesse, 1986) and is a major component of many common rock minerals. It is an essential element for plant and animal life. It is also a major component of most natural waters. In many bodies of water, calcium is present as $Ca(HCO_3)_2$, or $CaSO_4$, Gypsum (Stumm, 1992). When there is a pH change along with removal of CO_2 by photosynthesis, bicarbonate is converted to the insoluble calcium carbonate and precipitates out (Stumm, 1992). When conditions are anoxic, the reduction of sulphate to hydrogen sulfide by sulphate-reducing bacteria also leads to the precipitation of the original calcium sulphate as calcium carbonate (Bottrell, et al., 1993).

$$CaSO_4 + 8[H] + CO_2 = CaCO_3 + 3H_2O + H_2S \qquad [1.6]$$

The conversion of CO_2 to carbonate occurs by hydrolysis of NH_3 to NH_8OH, which dissociates partially to NH_4^+ and OH^-, thereby raising the pH of the environment to the point where some of the CO_2 produced may be transformed into carbonate. The cell then serves as a nucleus for further calcium carbonate precipitation seen as lamina on photomicrographs of thin sections of ooids (Mackenzie, and Adams, 1984). Bacteria in marine settings are recognized as possibly being involved in aragonite

($CaCO_3$) precipitation (Krumbein, 1974; Buczynski and Chafetz, 1990). Buczynski and Chafets demonstrated with samples collected from Bafin Bay, Texas, and from Andros Island, Bahamas, that bacteria not only act as nucleating material for precipitation of calcite and aragonite but that without the metabolic activity of bacteria, precipitation did not occur. It was discovered, however, that in the laboratory the precipitate from any given single culture is either aragonite or calcite, not a mixture of both. This seems to be related to the rate of precipitation. If precipitation is rapid, aragonite forms, and if slow, calcite. Bacteria which produce copious amounts of slime slow down the ion diffusion rate and as a result the precipitation rate is slow and calcite is the preferred mineral formed (Buczynski and Chafetz, 1990).

Cyanobactera are involved with the "whiting" phenomena seen to occur on the Great Bahama Bank (Boss, 1991). A similar whiting event occurs off the coast of Scotland but is caused by algae, *Emiliania huxleyi.* (Jong, and Vrind, 1997). The precipitation of carbonate can occur mostly on the outside of the bacteria but can be deposited intracellularly (Buchanan and Gibbons, 1974). The bacterium *Achromatium oxaliferum* has been reported to deposit internal carbonate (Ehrlich 1990). The carbonate deposits are caused by living as well as dead cells and therefore the process of $CaCO_3$ deposition by these micro-organisms is not dependent on the state of the cell (Greenfield, 1963). The amount of carbon incorporated into carbonate as a result of algal photosynthesis may be a substantial portion of the total carbon assimilated (Jensen et al., 1985).

The mechanism of $CaCO_3$ deposition is still not fully understood (Ehrlich, 1990) however Berner (1968) noticed that during bacterial decomposition of a fish in seawater in a sealed jar, calcium was precipitated not as $CaCO_3$ but as calcium soaps or adipocere (i.e., calcium salts of fatty acids) despite the presence of HCO_3^- and CO_3^{2-} species and an alkaline pH in the reaction mixture. Berner states that the fatty acid concentration favored calcium soap formation over calcium carbonate formation. Berner also suggested that in nature such soaps could later be transformed into $CaCO_3$.

Bacteria are not only involved in carbonate precipitation but are also, to a greater degree, responsible for the degradation or dissolution of carbonate (Ehrlich 1990). In nature carbonates, such as a limestone deposits, may easily be degraded or dissolved as a result of microbiological activity (Golubic and Schneider, 1979). The chemical reason for carbonate dissolution is the instability of carbonates in acid solution:

$$CaCO_3 + H^+ \rightleftharpoons Ca^{2+} + HCO_3^- \qquad [1.7]$$

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \quad [1.8]$$

$$H_2CO_3 \rightleftharpoons H_2O + CO_2 \quad [1.9]$$

$CaCO_3$ begins to dissolve in weak acid solution. Therefore, any organism that generates acid metabolic waste is capable of dissolving insoluble carbonates. Metabolic generation of CO_2 during respiration of organic matter may also be involved in carbonate dissolution:

$$CO_2 + H_2O \longrightarrow H_2CO_3 \quad [1.10]$$

and

$$H_2CO_3 + CaCO_3 \longrightarrow Ca^{2+} + 2\,HCO_3^- \quad [1.11]$$

Bacterial numbers found within limestone were quite sizeable; 18 x 10^6 per gram (Paine et al., (1933). Paine et al. believed that the numbers of bacteria found within limestone samples were in part dependent on the environment surrounding the stone. The surface of the limestone harboured higher numbers of bacteria than the interior of the stone. It was also found that bacterial populations were not evenly distributed and that populations tended to be higher in pockets, pores and interstices in the limestone structure. Experiments were performed (Paine et al., 1933) to estimate the rate of limestone decay through bacterial action under laboratory conditions. It was found that 0.18 mg of CO_2 per hour per 350 g of stone were evolved in one case, and 59 mg of CO_2 per hour per 350 g of stone in another. These results demonstrate that heterotrophic metabolism of organic matter was the cause of the observed limestone decay, autotrophic nitrifying and sulphur-oxidizing bacteria were also shown to be able to promote limestone decay through the production of nitric and sulphuric acids from ammonia and reduced sulphur, respectively (Paine et al., 1933).

1:5 Hydrological and Geochemical Controls in Cave Environments

Due to the high permeability of the Pleistocene limestones rainfall rapidly infiltrates into the subsurface, preventing the formation of surface rivers or streams in the Bahamas. Topographic lows that intersect the water-table are occupied by surface lakes and ponds. According to Vacher and Bengtsson (1990), when precipitation exceeds evapotranspiration, as in Bermuda, the groundwater lens is recharged by the overlying lakes. In the Bahamas, however, where evapotranspiration exceeds precipitation the lakes become evaporative discharge points for fresh water (Davis and Johnson, 1989). This results in the upconing of the salt water beneath the lens (Wallis et al., 1989). If evaporation continues, lakes become saline to hypersaline, a phenomenon commonly seen on islands such as San Salvador (Vacher and Bengtsson, 1990). Davis and Johnson (1991) demonstrated that these lakes force the fresh ground water to be partitioned into lenses beneath the high eolianite ridges.

Flowing surface water in the Bahamas occurs only in the creeks and bights which are connected with the sea and have low hydraulic gradients. What this means is that during high tide water flows inland via the creeks and, during low tide, flows seaward. Bights, on the other hand, cut through the island and have strong tidally reversing currents driven by differences in tidal head across the island. The principal fresh water aquifer in the Bahamas can be found in the highly permeable pre-Pleistocene Lucayan Limestone which truncates the base of the fresh water lens. Most of the morphology and hydrology observations concerning the nature of the fresh water lens and the associated mixing zone has been done on Andros Island (Palmer and Williams, 1984; Palmer et al., 1986) and Cat Island, Bahamas (Palmer et al., 1986).

Theoretical calculations and laboratory experiments have shown that when two solutions, each calcite saturated and with different salinities, are mixed, the resulting solution generally becomes undersaturated with calcite and, therefore, is capable of dissolving additional calcite (Hanshaw and Back, 1980). This is the geochemical explanation offered for the extensive dissolution and precipitation observed along the carbonate coast of the Yucatan Peninsula, Mexico (Stoessell, 1991) and the blue holes of the Bahamas (Whitaker and Smart, 1997, Smart et al., 1988) The theory is that the mixing of fresh ground water with subterranean sea water generates a highly reactive geochemical zone that enhances aragonite and calcite dissolution and permits neomorphism of aragonite. It is further believed that mixing zone dissolution, caused by ground-water discharge, is a major geomorphic process in developing caves (Hanshaw et al., 1986; Smart et al., 1988; Whitaker and Smart, 1997). It has been estimated that on the Yucatan Peninsula, the recharge waters annually dissolve about 37.5 metric tons of calcite per 1 km^2. When the groundwater has become saturated with calcite, little additional water-rock interaction occurs until the active mixing (dispersion) zone is reached near the coastline. Here, where both water types are

saturated with calcite but have a different salinity, the resulting solution is capable of dissolving additional calcite. Hanshaw and Back (1980) estimated that these now brackish waters are capable of dissolving an additional 1.2 mmol/L calcite, making these waters part of an important geomorphic process in areas with extensive limestone terrains.

It had been suggested by Smart et al. (1988), and Whitaker and Smart (1997) that cave development might not just be an abiotic process but, based on observation, biotic processes may be at work as well. Density gradients observed in the mixing zone within caves are believed to be caused by accumulation of particulate organic matter, providing an opportunity for bacteria to play a significant role in driving carbonate dissolution. Other observations such as bacterial growth on the walls and ceilings currently submerged in mixing zone waters provided additional evidence for bacterial presence.

1:5:1 Fresh Water Lens

In the Bahamas similar mixing zone calcite dissolution processes are at work (Whitaker and Smart, 1997). Diagenesis within the fresh water lens in the Bahamian carbonate platforms is believed to be driven predominantly by mixing (James and Choquette, 1984). Basically, the water body within carbonate platforms can be divided into three bodies; fresh water lens, the fresh-salt water mixing zone, and the deeper saline zone (Whitaker and Smart, 1997). In porous and relatively unconsolidated limestones fresh water entering the ground displaces the denser salt water and produces a lens-shaped body in accordance with the Ghyben Herzberg (GH) model (Vacher, 1988). Differences in the density of fresh water, saline water, the amount of recharge, and aquifer permeability will determine the shape of the fresh water lens. An extremely permeable aquifer will cause the fresh water lens to spread out over the saline water as a thin layer which often results in the formation of a brackish water layer on the underlying marine water (Vacher, 1988). However, should there be less lateral permeability, water, under hydraulic gradient, will move more quickly downward than it can move horizontally. This results in a fresh water lens, with a mound of fresh water developed above sea level (Vacher, 1988). In a state of equilibrium, the amount of water being lost at the edge of the lens (which can be seen as coastal and offshore springs or seeps), equals the amount of water filtering in from the surface, thus creating a stable but dynamic lens (Tarbox, 1987). However, the (GH) model is an over simplification of the true dynamics of the fresh water lens, as other factors such as the porosity, permeability variations, bedrock type, karst features, fresh water input, lakes, coastline configuration, and topographic features (e.g., eolianite ridges) all play a role

in the actual position, shape and thickness of the fresh water lens (Wheatcraft and Buddemeier, 1981; Ayers and Vacher, 1986; Davis and Johnson, 1989; Vacher and Bengtsson, 1990).

Resent research by Bukowski et al. (1998) does confirm that there is a problem with the (GH) model. Bukowski has discovered that the fresh water lens, contrary to the (GH) model, in the Pleistocene carbonate aquifer on North Andros Island specifically, is significantly thinner than the theoretical lens with depth below sea level approximately 10 times the head above sea level, as opposed to 40 times. This has serious implications as the fresh water lens is being pumped in excess of 1.8 million gallons above the 5.8 million gallon initially being pumped each day (Bukowski et al., 1998).

Another version of the (GH) model is the Dupuit-Ghyben-Herzberg (DGH) model. This model follows from the combination of the continuity equation and Darcy's Law with the (GH) principle and Dupuit's assumptions of horizontal flow. The (DGH) model is used to determine the position of the water table and salt water interface in island lenses in terms of island geometry, distribution of hydraulic conductivity, and distribution of recharge (Vacher and Bengtsson, 1990). Descriptively, meteoric waters pass through the vadose zone which in the Bahamas can vary from a few centimetres to a thickness in excess of 24 metres (Cant and Weech, 1986). Once through the bedrock, the rain water comes in contact with the water table. Here it is speculated that the meteoric waters are highly charged with PCO_2 and are mixing with degassing lens waters and initiating cavernous porosity (Equation 1.10 & 1.11) at the water table (Meyers, 1978; Longman, 1980). Five sub zones within the fresh water lens have been defined by Longman (1980. The division is based on decreasing hydrological and diagenetic flux with increasing depth below the water table and stagnation below sea level. The section of the fresh water lens below sea level accounts for over 97% of the lens volume according to the GH analysis. Petrographic analysis of Pleistocene carbonates suggests that in meteoric diagenesis of carbonate rock (Land, 1973) the phreatic zone is of more importance than the vadose zone. This theory is based on the idea that the fresh water lens had a longer residency time.

Examination of meteoric cements in fossil carbonates by cathodoluminescence show change from shallow oxic waters at the lens surface to reducing conditions within the main body of the lens (James and Choquette, 1988; Mussman et al, 1988). This has been observed in modern continental carbonate aquifers (Edmunds and Walton, 1983). Within the fresh water lens organic matter decomposition will occur either in oxic or in anoxic conditions. In anoxic conditions, sulphate reduction may generate a potential for carbonate dissolution (Coleman, 1985). This process has implications when considering young carbonate deposits which may have microbially available organic material trapped within composite grains by the cementation process.

However, according to Whitaker (1992) organically-mediated processes appear to be an important, if not the dominant, control of dissolution within the fresh water system, fresh salt water mixing zones and also saline zone. Whitaker also speculates that oxidation of organic material and possible *in situ* generated organics by chemolithotrophs generates significantly elevated levels of PCO_2. However, there is a problem with this theory in that chemolithotrophs utilize inorganic carbon (i.e. CO_2) and would play a role in reducing PCO_2 and not the reverse. Initial decomposition occurs within the shallow region of the water column by aerobic oxidation, but as this process consumes dissolved oxygen, conditions become anoxic and decomposition proceeds by sulphate reduction (see Figure 1.4). Both these processes, according to Whitaker, generate dissolutional potential. At this time, however, no general study has been conducted into the role of organic and inorganic oxidation in diagenesis of modern carbonate platforms.

1:5:2 The Fresh-Salt Water Mixing Zone

Wedged between the fresh water lens and the underlying saline waters is a zone of dispersion referred to as the mixing zone (Whitaker and Smart, 1997). This section of the water column is considered an environment for calcite dissolution (Back et al., 1979; 1986). However, at this time, the mixing zone diagenetic model is largely based on hydrodynamic modeling and experimental and theoretical geochemistry. Part of the problem of acquiring in-field data is that underground accessibility is limited in most regions of the world and, where it is possible to get underground, very few people care to do so. According to Cooper, (1959), active circulation within the mixing zone is developed by fresh water recharge and buoyant circulation. This water body then flows towards the coastal margin, causing compensatory inflow of sea water at depth. These models, however, do not predict what is occurring on modern carbonate platforms. Most models are validated using field mapping from coastal Florida (Cooper et al., 1964) and computer models (Cahill, 1967).

Cooper et al. (1964) and Cahill (1967) predicted that the mixing zone thickened towards the coastal margins due to larger volumes of water discharge through very permeable carbonates. Data collected from Bermuda supports these predictions (Vacher and Hearty, 1989) and data from atoll islands support the idea that tidal fluctuations play an important role as to where the mixing zone is positioned (Wheatcraft and Buddemeir, 1981; Oberdorfer et al., 1990). In other literature, however, the mixing zone is thought to be thinning towards the coast (Longman, 1980; Tucker and Wright, 1990). Radical dissolution of cave walls, walls having the characteristic "Swiss cheese" morphology, have been observed in both actively forming caves and in relic

caves found today 6 metres above current sea level (Back et al., 1986; Vernon, 1969; Mylroie and Carew 1990; Schwabe et al., 1993). Petrographic analysis reveals that preferential dissolution of aragonite occurs mostly within the more saline portions of the mixing zone (Stoessell et al., 1989).

1:5:3 The Saline Zone

Saline or marine water circulation can occur as a result of differences in fluid density (Whitaker and Smart, 1997), due to dilution by fresh water (buoyant circulation), or concentration of saline water due to evaporation and differences in water temperature. The other possibility of moving water around is by thermal convection (Figure 1.9). Differences in sea surface elevation across a carbonate platform may be caused by tides, local wave conditions, or ocean currents. Movement of tidal water around and through the carbonate platform may be restricted or slowed down by friction, creating a temporal lag and reducing tidal amplitude (Matthews, 1974). Wind and storms can control differences in sea surface elevation on leeward and windward sides of reefs (Atkinson et al, 1981) and islands (Hardie and Ginsburg, 1977). Although saline ground water circulation due to storm events has has been subject of speculation, direct observation during storm events show that when water is being pushed up onto the banks, the tidal cycle within marine blue holes can be reversed instantly (personal observation). When the storm has passed, the tidal cycle reverts back to the conditions prior to the storm (i.e., if the marine blue hole system is blowing prior to the storm as the waves are being pushed up onto the platform, it nevertheless begins to suck water in. When the storm has passed, the system reverts back to conditions prior to the storm, and begins to blow again).

Buoyant circulation may occur as a result of salinity variations within large bodies of water. Potential seaward flow of fresh water and the mixing with the underlying saline ground water may cause a compensatory inflow of sea water at depth according to Cooper, (1959). This process results in what is referred to as buoyant circulation (Figure 1.9B). Horizontal and/or vertical temperature differences between deep cold ocean water surrounding the platform and within the carbonate platform may be driven into circulation by geothermal heat (Figure 1.9D) as seen in Florida and by negative temperature gradients (Kohout, 1967; Kohout et al., 1977) in the Aegean Sea (Dando et al., 1995). Although there is speculation for large-scale saline ground water flow though carbonate platforms, direct evidence of the nature of the circulation is still unknown. However, Whitaker (1992) speculates from her field results that there exists a regional scale circulation system within the saline zone of the Great Bahama Bank. She further states that the process which is driving this circulation is the increase in

density on the bank surface due to evaporation which generates the potential for reflux (i.e., a return flow) (Figure 1.9C). There is also a second circulation system driving cold normal salinity sea waters through the platform which appears to flow from west to east, possibly driven by trans-bank differences in elevation head created by the Florida current, impinging on the west side of the bank. The various possible mechanisms driving circulation within a carbonate platform are summarized in Figure 1.9D. Geothermal heat together with evaporation on the banks and daily tidal movement of water through the island are most likely all responsible for driving circulation within carbonate platforms.

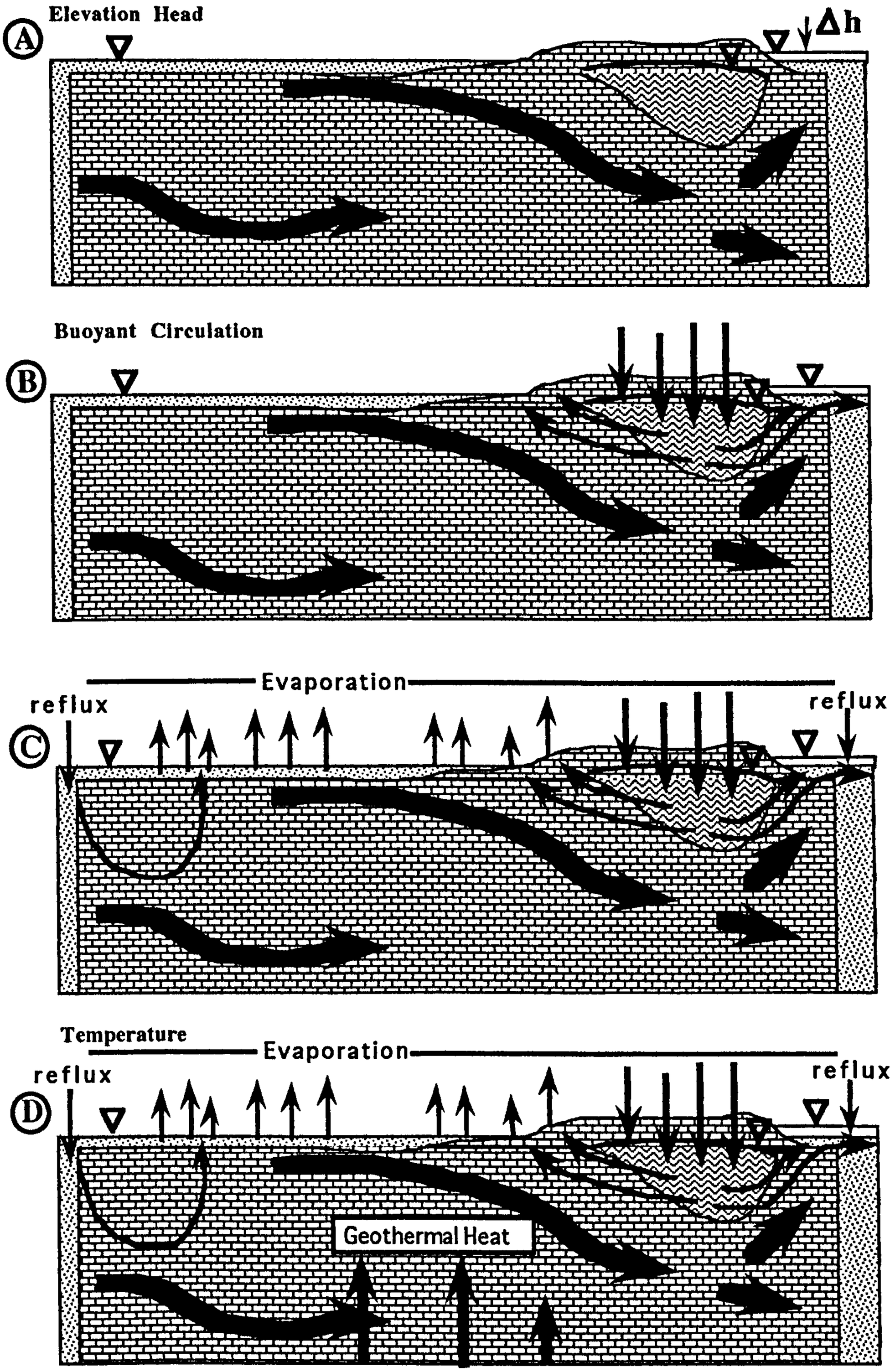


Figure 1.9 Diagrams illustrating the potential means by which water within a carbonate platform may be circulated. Diagrams B, C and D show combined processes from the previous diagram. Δh represents change in height of sea level (Partially redrawn from Whitaker, 1992)

2

Objectives, Experimental Approach and Choice of Field Area

The fundamental aim of this study was to investigate either directly, or via microbial impact on the geochemistry, the biogeochemical processes which are suspected of enhancing wall rock porosity and acceleration of cave development within and throughout modern carbonate platforms. Special emphasis will be placed on determining firstly whether bacteria are present in these environments, and if so, whether their numbers and activities are potentially significant enough to influence the water column and rock environment within the blue holes. Earlier work has led to the suggestion (Whitaker and Smart, 1997) that bacteria may be most active at the major density interfaces of the upper and lower mixing zone, and may also be responsible for specific geochemical effects which could not be explained through abiotic processes alone.

These objectives will be achieved by a number of field trips to Grand Bahama Island and South Andros Island. The Bahama Banks are the largest known modern-day carbonate platforms in the world and provide a natural laboratory for examining modern karst processes within extensive networks of flooded caves (blue holes); karst features which allow direct access to the ground water within the interior of the platforms.

2:1 Experimental Objectives

When considering the experimental objectives, due consideration had to be given to the fact that the aqueous cave environment limits diving and therefore limits the methods which could be used. With these limitations in mind, the experimental objectives were:

1) To determine whether bacteria were present, and if so determine whether bacterial numbers were significant, i.e., significant enough to potentially alter the environment.

2) To determine the distribution of bacteria and their activity in the cave system and hence define the specific cave conditions where bacterial processes are significant

3) To determine the presence and distribution of bacteria in the wall rock and sediment.

4) To provide evidence by demonstrating direct bacterial involvement in calcite dissolution and hence cave developement.

Objectives were to be achieved by:

1) Using specialized collection techniques to preserve *in situ* separation of water bodies and mixing zones over short vertical distance.

2) Analysis of water samples for depth distinctions of:
   a) conductance versus depth profile,
   b) temperature versus depth profile,
   c) dissolved oxygen,
   d) pH and alkalinity,
   e) particulate organic matter (POM),
   f) dissolved organic carbon (DOC).

3) Microbiological analysis of samples for:
   a) bacterial enumeration using direct count procedures
   b) radioisotopic tracer studies to measure bacterial activity.

4) Collection and analyis of cave rock cores for:
   a) thin-section petrological analysis,
   b) determination of the presence of bacteria,
   c) incubation studies.

5) Collection of evidence indicating direct bacteria involvement in cave diagenesis:
   a) rock digestion studies,
   b) SEM microphotograph,
   c) incubation studies using pH indicators to observe localized pH changes on rock core surfaces.

2:1:1 Criteria for and Selection of Study Area

The study area for this project needed to fulfill several criteria:

1) presence of a fresh water lens,
2) presence of a mixing zone,
3) presence of straight-forward access to a section of the cave which was not too deep into the cave system and could provide, in one area, a full range of the different bodies of the water column, i.e., fresh water/mixing zone/marine water,
4) accessible location,
5) the hydrology of the system needed to be relatively well understood and not too complex and
6) evidence was needed to suggest bacterial presence and activity at the location.

This led to the selection of 3 caves in the Bahamas for this research project:

1) Lucayan Caverns and Owl's Hole are both lens-based (phreatic) caves with apparently similar geochemical and hydrological processes. The biggest known difference between the two systems is that the Lucayan Caverns cave system is hydrologically directly connected to a mangrove creek whereas Owl's Hole is not. Sampling two adjacent lens-based caves would enable comparison of similarities and differences between the two and possibly the effect of the mangrove creek.

2) Stargate is a fracture system, [refer to section 2.6],which would provide an effective comparison to the other sites.

3) Previous research on the geochemistry and hydrology of all three systems (Whitaker and Smart, 1997) would provide for comparison a data framework to build upon and would also indicate presence of bacterial activity.

4) Lucayan Caverns are situated in a National Park, managed by the Bahamas National Trust, who allow only permit-holding divers into the system and therefore limiting the diving traffic within the cave. Owl's Hole and Stargate tend to be dived slightly more frequently than Lucayan. It is not yet fully

understood what the effects on the caves are when subjected to regular diving traffic, hence, limited diving and thus limited disturbance was an asset.

5) These three cave systems are generally representative of most of the known cave systems in the Bahamas.

Several locations offered at least some of these necessary criteria, but were for various reasons less suitable for this project. The Yucatan Peninsula in Mexico, which has extensive underwater cave systems, mixing zones, and a fresh water lens (Hanshaw, and Back, 1980) is readily accessed, but has a hydrological system with a higher flow regime when compared with static lenses such as those found in the Bahamas. The Nullarbor Desert in Southern Australia is another area which has extensive water-filled cave systems in its subsurface. These systems have a perched brackish water lens, they have a mixing zone, the hydrology of the system is relatively simple and at least partially understood, and some bacterial activity has been indicated (James et al., 1989). However, the environment is extreme, i.e., wide temperature ranges and unpredictable precipitation, and without any detailed background knowledge concerning bacteria in caves in Australia, it seemed wiser to try to sample an area were it was observed that bacteria were present (Whitaker and Smart, 1997). Two of the Bahama caves are located in Grand Bahama, and one on South Andros. A description of these caves will be given in the text, along with a general description of the geology of the Bahamas, including factors which influence the subsurface environment such as climate and vegetation. A description of general cave macro fauna will be included, because macro fauna play a role in organic cycling within these subsurface environments and, deeper in the caves, may rely on bacteria as an important food source.

2:2 Geographical Considerations

The Bahamian Archipelago, which is located between 20° to 28°N and 72° to 80° W, consists of an area approximately 800,000 square kilometres and supports 11,406 square kilometres of subaerially exposed land and 125,000 square kilometres of shallow bank (Meyerhoff, and Hatten, 1974). The homogeneous carbonate platforms and shallow banks are dissected by deep troughs. Platform water depth is generally less then 15 metres and the troughs, such as the Northeast Providence Channel, have depths up to 4000 metres (Meyerhoff, and Hatten, 1974).

2:2:1 General Geology and Landscape Topography

The Bahama banks began to form some 200 million years ago, at about the same time as the North Atlantic. The islands are composed of modern-day carbonate which are virtually identical in composition to carbonate material recovered from water depths of 6,100 metres (Sealey, 1994). What this suggests is that the rock material laid down at the beginning of platform development formed under the same (or similar) conditions as today. Virtually the entire platform is composed of pure limestone. Through changing depth, however, the composite grains which make up the rock will vary (Eberli and Ginsberg, 1987). Except for the occasional iron oxyhydroxide deposit, the limestone is composed of organic benthic forams, algae and shell hash (Schwabe et al., 1993). The inorganic fraction is composed of ooids and peloids (Dix and Mullins 1988; Gaffey 1988). Ooids and peloids [refer to Table 1:3] can also contain particulate organics, which can act as nucleating material for aragonite or calcite. It is not uncommon to find small shell bits, fecal pellets or bacteria in the core of the ooids and peloids (Schwabe et al., 1993).

The composition of the basement of the Bahamian carbonate platform is still unknown. Geophysical-seismic results indicate properties that are transitional between continental and oceanic crust (Malfait, and Dinkelman, 1972). A 5,443 metre core recovered in 1971 from Great Isaac's Well, an area just north of Bimini, was shown to be composed of limestone and marine shales from the early Jurassic Period along with some volcanic debris (Austin and Schlager 1986). Bimini is a series of small islands located approximately 85 km due east of Miami, Florida. It has been suggested that the volcanic material may have come from the North American plate (Mullins and Lynts, 1977). According to Mullins and Lynts, sections of the African continent were rifted during the Triassic/Jurassic opening of the North Atlantic. The transitional properties of this crust are thought to be due to continental crust that was pervasively intruded by mafic and ultramafic magmas during periods of active rifting (Diet et. al., 1970; Lynts, 1970). It has been proposed that in order to solve the problem of the pre-rift overlap, reconstruction can be achieved by rotating the archipelago 25^0 to the Northeast (Mullins and Lynts, 1977).

The geological events which were responsible for the production of the Bahamian banks and invasive troughs are still a subject of deliberation. Mullins and Lynts (Mullins and Lynts 1977) suggested an initial Horst and Gräben topography that was created during the Pangea rifting event in the Mesozoic. Considerable carbonate depositions on the Horste and less in the deeper Gräben is suggested to be responsible for the existing platforms and deep channel topography (Mullins and Lynts 1977). In contrast, the "megabank" hypothesis proposed by Schlager and Ginsberg (Schlager,

W. and Ginsberg 1981), and by Sheridan and others (Sheridan et. al., 1981) suggests that one large bank was formed during the Jurassic. Eventually, the present form of the banks and troughs was produced in the Cretaceous by gravity-flow, headward erosion, and scour and fluctuating rates of sedimentation. There are, however, arguments against the "megabank" hypothesis by Eberli and Ginsberg (Eberli and Ginsberg, 1987) which are supported by a detailed seismic study of the north-western Great Bahama Bank. Eberli and Ginsberg have proposed that the current configuration of the bank developed due to the coalescence of three smaller platforms and subsequent lateral progradation. Progression up to 25 kilometres into former channels has been interpreted from seismic reflection profiles. Mullins and Hine (Mullins and Hine, 1989) have suggested, from results collected using Landsat imagery and seismic reflection profiles, that platform margins are actively being reduced in size by catastrophic collapse. Scalloped margins, which typify this event, can be seen to occur predominantly in the south-eastern area of the Bahamas. The morphology of the Bahama platforms is the result of a complex interaction of bank growth and retreat, superimposed on an existing tectonic framework since the Mesozoic.

Although the Bahamian islands have been considered technically stable, Mullins and Lynts (Mullins and Lynts, 1977) have demonstrated that the platforms are slowly subsiding. Garrett and Gould (Garrett and Gould, 1984) have estimated the rate of subsistence for Andros Island and the northern Bahamas to be approximately 24 m per million years. Carew and Mylroie have demonstrated similar rates to those estimated by Garrett and Gould for the entire Bahamian Archipelago (Carew and Mylroie, 1992).

Topographically, the Bahama islands can be divided into four different zones: the ridge lands, the wetlands, the rockland and the coastland (Sealey, 1994). The ridges range in heights on different islands between 10 to 45 m above sea level, with a few less than 5 m high. Ridges can range in length from several kilometres, again depending on the size of the island. Petrological examination of the ridges on Andros, New Providence, Great Inagua, Long Island, and San Salvador reveal that the most common compositional makeup of the interior of the ridge is bioclastic with overlain ooid deposits. One exception to these findings is on Long Island where one ridge has consecutive ooid deposits with an unconformity, marking the end of the initial deposit (Schwabe et al., 1993). Interior investigation of the compositional makeup of the ridges was achieved via fossil phreatic caves, commonly referred to by Mylroie and Carew (Mylroie and Carew, 1990) as flank margin caves. Openings to these caves can be found at 6 m elevation along the edges of ridge deposits (Mylroie and Carew, 1990). These currently dry caves formed during the late Pleistocene when sea level was 6 m higher than today (sub stage 5e, ~125,000 ca), and had a small fresh water lens within

the eolianite ridges. These large voids (14,000m^3) formed over a short geological time (< 15,000 years) (Mylroie and Carew, 1990).

Wetlands in the Bahamas can be found in the interior of the island in some cases, as hypersaline lakes and brackish lakes, and along the coast as tidal flats. The interior lakes on San Salvador Island, for example are hypersaline (47 0/00 salinity) (Pentecost, 1990). In these lakes, higher plant growth is restricted by high salinity or sediment instability (i.e., sediment too soft to support any significant amount of weight) and, more often than not, cyanobacterial mats and stromatolitic algal heads are characteristic features (Neumann et al., 1988).

The coastal tidal flats often have tidal creeks running through them. On Grand Bahama such a creek is known to be hydrologically connected to the "Lucayan Caverns" (personal observation). Numerous tidal creeks can be found on Andros Island and the eastern end of Grand Bahama. These larger creeks are referred to as "bights" (Palmer, 1995). In some cases, these creeks can contain fresh water despite the fact that they are connected to the open sea. Fresh Creek located on Andros Island is such a creek. It becomes fresh at low tide when the fresh water lens drains out via the creek bed.

The rock lands of the Bahamas have a very varied topography. The highly altered surface is inundated with dissolution features such as solution pits called "banana holes", but also locally called "pineapple holes" and "potholes". Some of these pits vary in depth from 1 m to several metres. In some areas the surface rock has collapsed into small conduit systems which have dissolved the supporting rock away a few centimetres beneath the surface. These features commonly start near ridge tops and can be followed to the base of the ridge where these conduit systems sometimes drain into lakes. When the salty water of the lake mixes with the meteoric waters draining off of the ridges it causes the rock in this immediate setting to dissolve much more readily, [refer to section 1.5], creating a feature called phyto-karst, sometimes referred to as "moon rock". These needle-shaped rocks can also be found in the spray zone along the coast where rain water mixes with sea spray.

In areas where the rock is void of vegetation, a limestone crust has developed. This crust is referred to by different names such as caliche or flint rock. Blue holes are another primary solutional feature commonly found throughout the Bahama Islands but will be discussed in greater detail later.

2:2:2 Climate and Vegetation

The Bahama islands lie on the boundary between the temperate and the tropical zones. The summers are wet and the winter season relatively dry. The Bahamas receive rain all year round. The average maximum temperature is between 25°C to 32°C during the summer months, with an average minimum of 17°C to 24°C during the winter months (Sealey, 1994).

The north east trade winds, which blow all year round, have their patterns altered frequently during the year either by cold fronts which originate from North America during the winter months or by hurricanes from the Southeast Atlantic in the summer months. The Bahama islands cover a great latitudinal distance, 730 kilometres, stretching from 21°N to 27.5°N. This makes it necessary to split the Bahamian archipelago into two biogeographical regions; a wetter, northern region with an annual rain fall of 60 inches / 1524 mm and a covering vegetation consisting of pines and broad leaf scrub, and a dryer southern region with an annual rain fall of 30 inches/ 752 mm (Sealey, 1994) and a vegetative cover consisting of broad-leaf deciduous shrub. Climate and vegetation may historically dictate, to a degree, the type and size of cave which may develop within the carbonate platforms (Sealey, 1994; Smart and Whitaker, 1988). It is known that rain is the major direct control on the rate of carbonate dissolution because of its effect on the net groundwater flux (Smart and Whitaker, 1988). It is also known that soils produced by dead or dying vegetation contribute to the production of carbonic acid. Carbonic acid, formed by carbon dioxide generated by microorganisms and root respiration in the soil, dissolving in water provides the major chemical potential driving dissolution in limestone areas (Smart and Whitaker, 1988). Humidified soils and organic soils contain high CO_2 which acts to dissolve the carbonate rock (equations 1.10-1.11) (Smart and Whitaker, 1988).

2:2:3 Blue Holes

Blue holes are entrances into some of the world's most spectacular underwater cave systems. There openings are among the shallow creeks, inland lakes, and the shallow banks of the Bahamas. The caves which have developed within the Bahamian carbonate platforms can be laterally and vertically very extensive. Lateral cave passages can extend to several kilometres and vertically blue holes may range in depth from 10 m to several hundred metres (personal observations).

For many years, ideas about the origin of blue holes have been mixed with local superstition and myth. Blue holes are sometimes referred to by native Bahamians as

"blowing" or "boiling" holes, a phenomenon supposedly created by a mythical creature called the "Lusca"; a belief still shared by many Bahamians today. The "blowing" and "sucking" phenomena are largely caused by differences in water surface elevation across the Bahamian platform generated by tides, local wave action, and ocean currents (Whitaker and Smart, 1997).

Bahamian caves can be broadly classified into three types: (1) lateral phreatic systems, (2) cenote, and (3) fracture-controlled. Division into these categories is based on the primary morphological controls during their course of development.

In theory, **Lens-based cave; lateral phreatic systems** (Figure 2:1 (A)) form along particular horizons where the interface between fresh and saline waters is *in situ* long enough for such complex passage networks and chambers to form. There is some evidence that suggests that preferential dissolution may take place along beds composed primarily of bioclastic sediments (Schwabe, et. al., 1993), but the key requirement is for a stable lens base and associated biotic input to accelerate chemical dissolution processes.

Fracture-controlled (Figure 2:1 (B)) caves differ from the lateral phreatic caves in that the morphological control is vertical rather than horizontal, allowing the movement of the mixing zone during glacio-eustatic vertical migrations of sea level to dissolve fissure walls and enlarge cave passages by dissolution and resultant gravitational splitting when the buoyant support of the water table is removed.

Cenote caves, (Figure 2:1 (C)) which can be found both inland and in a marine setting on the carbonate bank, may be created by a mixture of the above two controls when a pre-existing void, formed by lateral phreatic development of fracture dissolution, suffers roof collapse during low sea levels, perhaps associated with local fracturing of the host rock which, following the collapse, migrates to the surface. Voids created in this way are typically bell-shaped (personal observation), with the base wider than the surface opening and may be several hundred metres deep. Water movement is generally restricted in these systems and in many instances several very distinct thermoclines, haloclines and chemoclines can be found within the water column. Water within cenotes can be chemically holomictic however, because of microbial activity, become stratified by thermal gradients which vary in intensity based on the seasons.

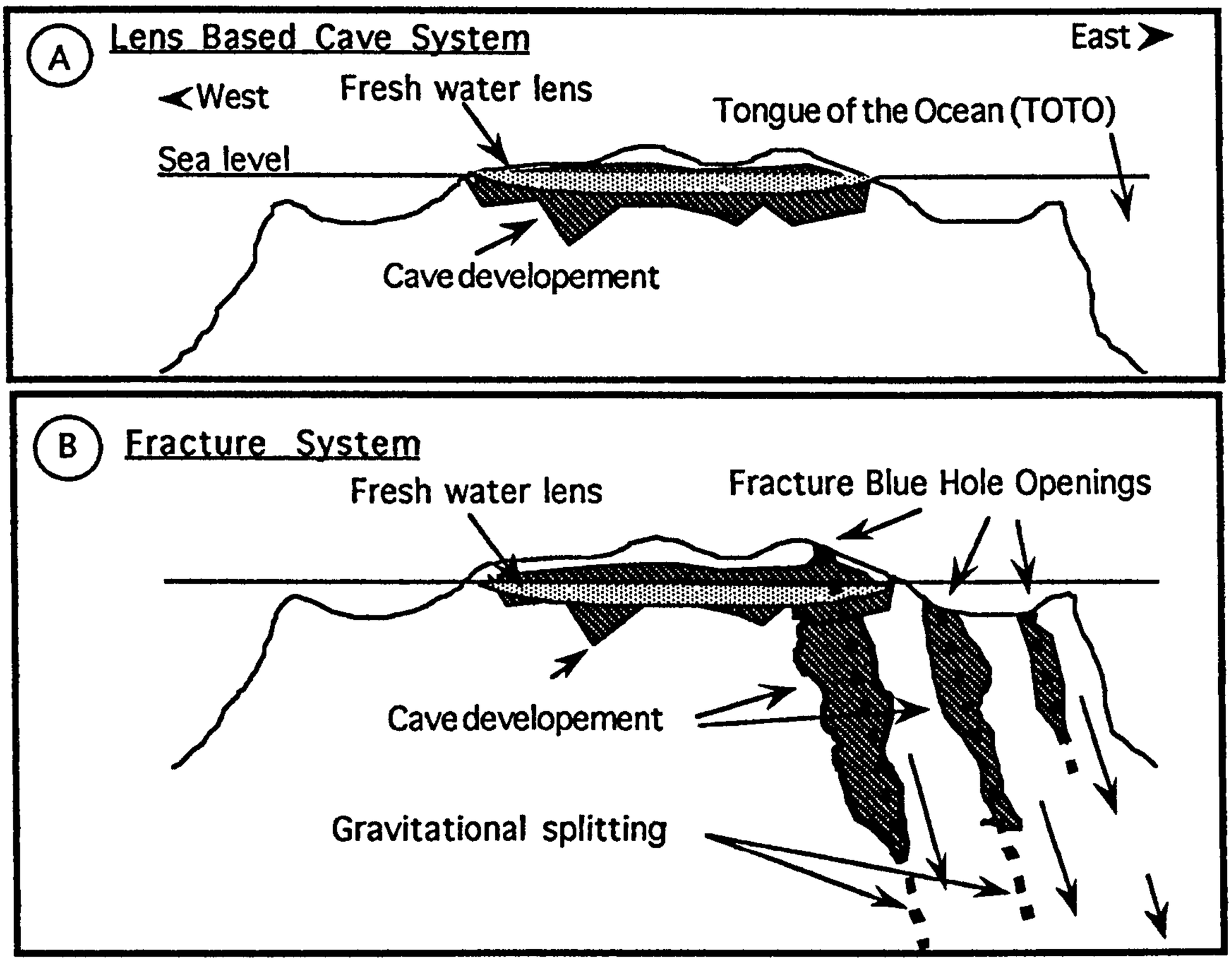


Figure 2:1 : Broad classification of caves found within the Bahamas. (A) lens based cave, sometimes referred to as lateral phreatic systems; (B) fracture controlled cave development; (C) cenote cave development; possibly controlled by paleo-reefs.

2:2:4 Cave Macro fauna

A variety of cave life occupies inland and marine blue holes; a great deal of it depending very much on microbial-based life (per observation), the subject of this thesis. The most visibly striking and well established of these niches are the entrances to the marine blue holes, an environmental setting which is not exclusive to the Bahamas but which can also be found in Bermuda around lava tubes (Iliffe, 1986) and in other marine cave settings. Around and immediately within the entrances, referred to as the "arena," (Palmer, et. al., 1986) large populations of crustaceans, sharks, numerous species of fish, molluscs, polychaetes and other invertebrates can be found sheltering within the cavern. Dense coral growth typically fringes marine holes in sea water depths of over 3 m.

Moving down the throat of the cave, the "vestibule", (Palmer, et. al., 1986) into the beginning of the aphotic zone, referred to biologically as the "transition zone", (Palmer, 1997) the wall fauna begins to slowly change from daylight species to encrusting bryozoans, hydroids, serpulid worms, sponges and numerous other filter feeders, although gorgonian corals and ahermatypic corals can be found several hundred metres back inside a cave which is unusual as they are thought to need sunlight to survive. It is also not uncommon to find large fish and sharks deep within the cave system.

In deep marine cave systems just recently explored on the South Andros fracture, which have depths in excess of 100 m (Palmer, 1996), it has been observed that at approximately 60 m there is a noticeable thermocline, below which the walls become virtually devoid of visible marine life, save for occasional serpulid tubes or infrequent small sponges. This can be seen to continue throughout the explored passage of the deep cave zone (Palmer, 1997). The tidal currents entering the cave appear to flow laterally at shallower depths, above 60 m. Within the deeper passages, cooler up-welling waters (Palmer, 1997) have been observed, but because of the extreme depth and distance from the entrance, investigation of these sites are difficult. Several hundred metres into these systems, marine life becomes visually increasingly scarce as food supplies dwindle, and this "deep cave" zone (Palmer, et. al., 1986) is typified by a more cavernicole fauna which may also be present below the 60 m chemo- and thermocline.

Most interesting, however, are the discoveries in recent years of "living fossils", (Iliffe, 1986; Yager, 1981) and deep sea species within these cave systems (Iliffe, 1986; Kornicker and Iliffe, 1998). Species, which are numerous, have been found in cave environments which appear older than the cave they inhabit, while other species show anomalous and widely separated zoo geographic relationships to present

day deep sea taxas (Iliffe, 1984). These findings indicate that not that many fresh water cavernicoles have a marine origin (Iliffe, 1984).

2:3 Historical Perspective of Blue Holes in the Bahamas

Caves in the Bahamas, both above and below sea level, were described in literature dating as far back as 1725 by an English naturalist, Mark Catesby (Shaw, 1993). Further interest in these caves was generated by the exportation of guano to the Americas and Europe. The underwater caves, (Blue Holes) were first recorded on sea charts at about 1843 (Shaw, 1993). Ten years later, Captain Richard John Nelson began geological studies in the Bahamas. He noticed the fluctuating levels of brackish water in the banana hole (solution pits) and was the first to recognize that the fresh ground water formed a lens and floated on top of salt water (Shaw, 1993) and that the source of the fresh water was rain.

In 1890, geologist Dr. John I. Northrop spent nearly half a year in the Bahamas exploring and studying inland and marine blue holes (Northrop, 1891). Ocean holes were also explored by an oceanographer and zoologist, Alexander Agassiz (Agassiz, 1984). He studied the Bahamas and the surrounding sea bed in the early part of 1983 and was interested in the information provided by the blue holes concerning subsidence of the land.

Agassiz (Agassiz, 1984) also discussed in his writing how blue holes may have formed. He assumed that the conditions for cave development in the aeolian strata were the same processes which had formed potholes, boiling holes, banana holes (solution pits), caverns, caves, sinks, and other such openings on the shores of islands. He speculated that these features were formed by the action of rain percolating through the aeolian rocks and becoming charged with carbonic acid, or rendered acid by the fermentation of decomposed vegetation or animal matter or by the action upon the limestone of sea water or spray under the most varying conditions of elevation and of exposure. He believed that ocean holes formed under very much the same conditions when the submerged bank was above the high water mark.

Charles Johnson Maynard, a taxidermist and naturalist from the USA, made five collection trips to the Bahamas (1894) and published his results in Contributions to Science (Shaw, 1993). Maynard had the idea that marine blue holes passage genesis was controlled by the space left when coral was forming into reefs and that they were kept open by water flow or even enlarged by the dissolving away of the dead coral before they were later buried by sediments. He believed that no matter how thick a coral reef was, it still did not form a solid wall, but was honey-combed in all directions

by passages which are either left through the irregular method of the formation of the coral heads and branches or from dissolving away the dead coral.

Several others followed Maynard in their quest to interpret the geology and natural history of the Bahamas, such as Moseley in 1926 (Shaw, 1993), who describes the finding of a skeleton of a Lucayan Indian on the island of Nassau. Then in the 1950's, a diver by the name of George Benjamin began exploring the underwater cave systems, using a self-contained underwater breathing apparatus (SCUBA) (Benjamin, 1970). Benjamin is credited with being the first to describe these caves from within. Benjamin believed that cave development occurred during times of low water when the ocean waters were trapped as ice during the ice age. He speculated that when the caves were dry, the slightly acidic rains ate away the basic limestone, opening fissures and forming underground pockets. Flowing water followed the meandering crevices and enlarged them, turning the pockets into caverns (Benjamin, 1970).

A photographer by profession, he produced watertight housings for his cameras and was able to begin the first documentation of these underwater caverns. After many years of exploration and the loss of several of his friends and colleagues to the cellars of the sea, (as Benjamin used to refer to them) he stopped his explorations. At this time, a young British cave diver, by the name of Robert John Palmer, became interested in Benjamin's stories of these amazing blue holes and contacted him, asking if he could come on one of his trips. Benjamin told him that he was no longer going to be exploring these sites and that Palmer was more than welcome to all of the information concerning the blue holes that he had. Palmer picked up where Benjamin stopped in 1977, and for nearly a decade explored and documented his adventures in books, films, various scientific journals, and as co-author of numerous papers.

2:4 Description of Lucayan Caverns, Grand Bahama

Lucayan Caverns is one of the most extensive lens-based cave systems known in the Bahamas (~10 kilometres of mapped passage), and is located on the southern side of Grand Bahama (Figure 2.2). It is about 32 kilometres east of Freeport, just off the main highway running to the Gold Rock Creek Tracking Station, which is about 5 kilometres east of Lucayan National Park. There are three known "dry" entrances and two known "wet" entrances to the system, which discharge into, and recharge from, Gold Rock Creek, a mangrove creek connected directly to the sea.

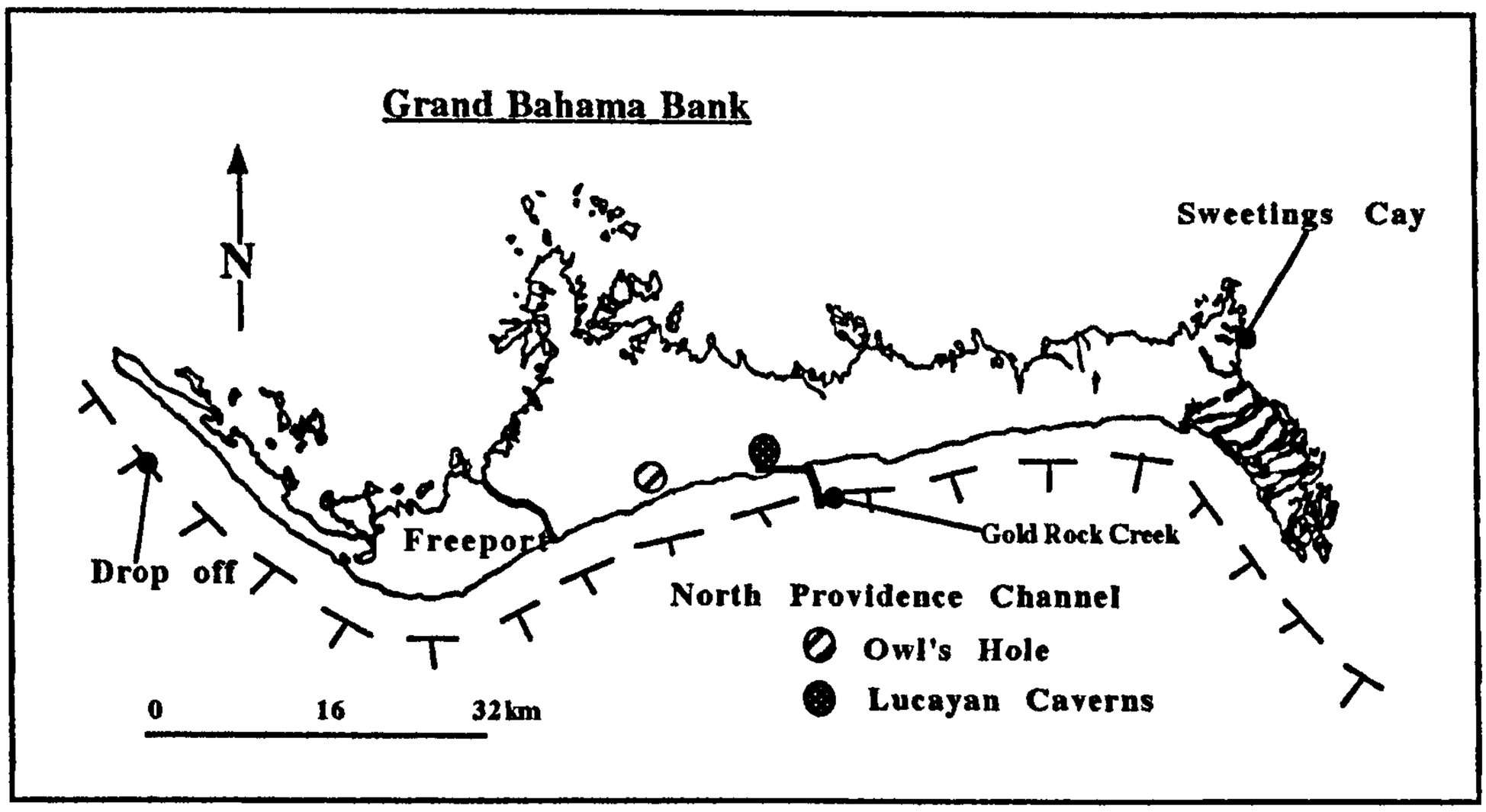


Figure 2.2: Map of Grand Bahama showing the location of both the Owl's Hole and Lucayan Caverns site.

The main "dry" entrance, Ben's Cave, has formed where a collapsed cavern ceiling has created a "cantilevered arch or dome" (Palmer, 1981). This cavern is also one of the breeding and maternity sites for the buffy flower bat (Erophylla sezekorni) (Andersen, 1990). This bat is also found in similar settings in Lighthouse Cave on San Salvador Island and in a drier setting, the Alter Cave, also on San Salvador Island. These bats represent an important source of nutrients (guano) for the cavern waters as well as supplying the Mangrove Snapper (Lutjanus griseus) population with the occasional baby bat which falls to its death in the waters below.

The waters in Ben's Cave; (GPS coordinates: 26° 36.325 / 78° 24.091) start out fresh at the surface and progress through a sharp mixing zone at 13 m into the deeper marine waters. The average depth throughout the Lucayan system is about 16 to 19 metres. From here the passages eventually connect with the other two entrances, beyond which a complex network of primarily phreatic passages lead into the interior of the island through a maze of stalactites and stalagmites and occasional solutional or collapse chambers. One particularly distinctive passage, named Avalanche Alley, has a distinctly different morphology than the rest of the cave. Visually, the passage is one long north-south collapse feature, following the bedding planes of the rock. Petrological examination revealed that the rock was composed of unusually large, (personal observations) very well-rounded peloids. These may have formed in a back beach lagoonal setting. The cave itself has developed mostly within the Lucayan limestone which almost exclusively is composed of bioclastic composite grains.

The next large dry entrance is called "Indian Burial Mound Entrance" so named because of the Indian burial mound found at this site; (GPS: 26° 36.365N / 78°

24.100W). This particular entrance is much larger because of ceiling and lateral collapse into the cave opening. The third entrance, Skylight Room, is located about fifty metres east from the Burial Mound entrance, and is not normally entered from the surface, it being a fairly long drop to the water below. The room below this entrance can be entered by snorkeling from the Burial Mound entrance.

The "wet" entrances, which hydrologically tie Lucayan Caverns to the tidal creek, lie in Gold Rock Creek within the mangrove swamp. Both entrances, which are adjacent and connected to one another, provide a direct hydrological access to the main system for large amounts of particulate organic material (POM) from the creek.

Most of the passages in the system lie at or near to the present level of the active mixing zone, though all show indications of previous active development prior to the current post-glacial sea stand. Speleothem development reveals several successive exposure and inundation cycles, and there is some visual evidence of vadose downcutting in the further reaches of the cave. The cave appears to be more hydrologically active in its western passages, possibly due to the immediate influence of the creek waters, and deeper passages to the east are virtually backwaters. The cave immediately to the north of the "dry" entrances is essentially a complex, interconnected series of bedding chambers, breaking out into larger conduit passages in the extreme north of the explored system. At the far northern extremities of the cave, water flow issues from low bedding passages intersected by the mixing zone.

2:5 Description of Owl's Hole, Grand Bahama

Owl's Hole (so named because of the resident barn owl), like Lucayan Caverns, is located on the south side of Grand Bahama, approximately 4 kilometres due west of the Lucayan National Park (Figure 2.2);(GPS coordinates: 26° 35.273N / 78° 28.163W). The entrance to Owl's Hole is a circular 20 m wide opening with a sheer 6 m drop to the water surface. This cenote-type cave entrance is entered by using a caving ladder secured to the surface.

Owl's Hole and Mermaid's Lair, another opening (GPS coordinates:26° 35.175N / 78° 27.814W), are linked by a series of passages forming a complex horizontal maze, but which also extend into other directions below the island to the north and east. The distance between the two entrances is approximately 1000 metres. Owl's Hole appears to be a major conduit system following a depth around 16 to 20 m, lying mostly below the current mixing zone, which is evident upstream only in the passages between the entrance to Owl's Hole and the sampling site of Paul's Palace, and again in the Broken Arrow- Hume's Hall area near the present northern end, 700

metres from the entrance. The collapse of the roof has again created a sizable chamber reaching up into the fresh water lens. The mixing zone is extremely thin at this point. Most of the rest of the system lies below the mixing zone, and is typified by large, bedding-influenced passages 2 to 3 m across and 1 to 2 m high. Formations (i.e., stalagmites and stalagtites) are relatively scarce, and walls, roof and floor are coated with dark brown flocculent material. Where the mixing zone is visible, flow is evident in a southerly direction. Strong current flow may be occasionally present, suggested by the burial of scar marks caused by diver traffic in the floor sediment before Paul's Palace.

Downstream from the Owl's Hole entrance cenote, two passages lead to and combine in a shallow fresh water chamber above the mixing zone. This continues as a large, shallow collapse passage with one major upward collapse that goes almost to air space before dropping again into phreatic maze near Theo's Room. Current flow is evident in the mixing zone in this area, and a complex series of passages containing breakdown (i.e., rock collapse material) lead to the Big Room and the junction with Mermaid's Lair passages. The cave alters at this point, with current flow moving south towards the Applesauce Hill area, presumably to flow into the sea nearby. Brackish water has been reported as rising from sand flats just offshore (Ben Rose, personal comm.) and this is presumably the "resurgence" for the system.

The complex series of maze passages continue eastwards towards Mermaid's Lair, with little visible POM, and below whatever fresh water lens may exist above. These passages are well decorated, (i.e., lots of stalagmites and stalagtites) with clear visibility and floors of carbonate sand and rock. Below the sand covering, beds of orange muds can be found. In the region of the Mermaid's Lair entrance, a layer of organic black material is sandwiched between the muds and lime sand covering, penetrating for several tens of metres into the cave. Branches and twigs in this may indicate a period of geologically recent inflow into the system from this entrance. Speleothems in this region are profuse, and appear to indicate successive periods of emergence, with old corroded flowstone being overlaid by fresher deposits. The massive entrance cavern of Mermaid's Lair itself contains a very thin lens (~3m) immediately below the entrance pond.

2:6 Description of Stargate, South Andros Island, Bahamas

Stargate, which is located behind the Bluff, South Andros (Figure 2.3 and Figure 2.4), formed on the major N-S fracture line which parallels the edge of the Bank; (GPS coordinates: 24° 06.051N / 77° 33.081W). The entrance to this cave lies beneath a

cavern overhang, with a drop to the water level of ~ 4m. The vertical water column, directly below the entrance to Stargate, is relatively clear, except for a layer between 10 metres and 20 metres. Here the water column contains a white, wispy, undulating layer of clouds. This layer is associated with dramatic water chemistry changes over a vertical distance. There have been rare occasions when this layer appeared ordered, with the material appearing to have organized itself into a gill net. These layers are rapidly dispersed by divers, but are restored to the cloud form within 24 hours. The white cloudy material is most likely bacterial in composition (personal observation).

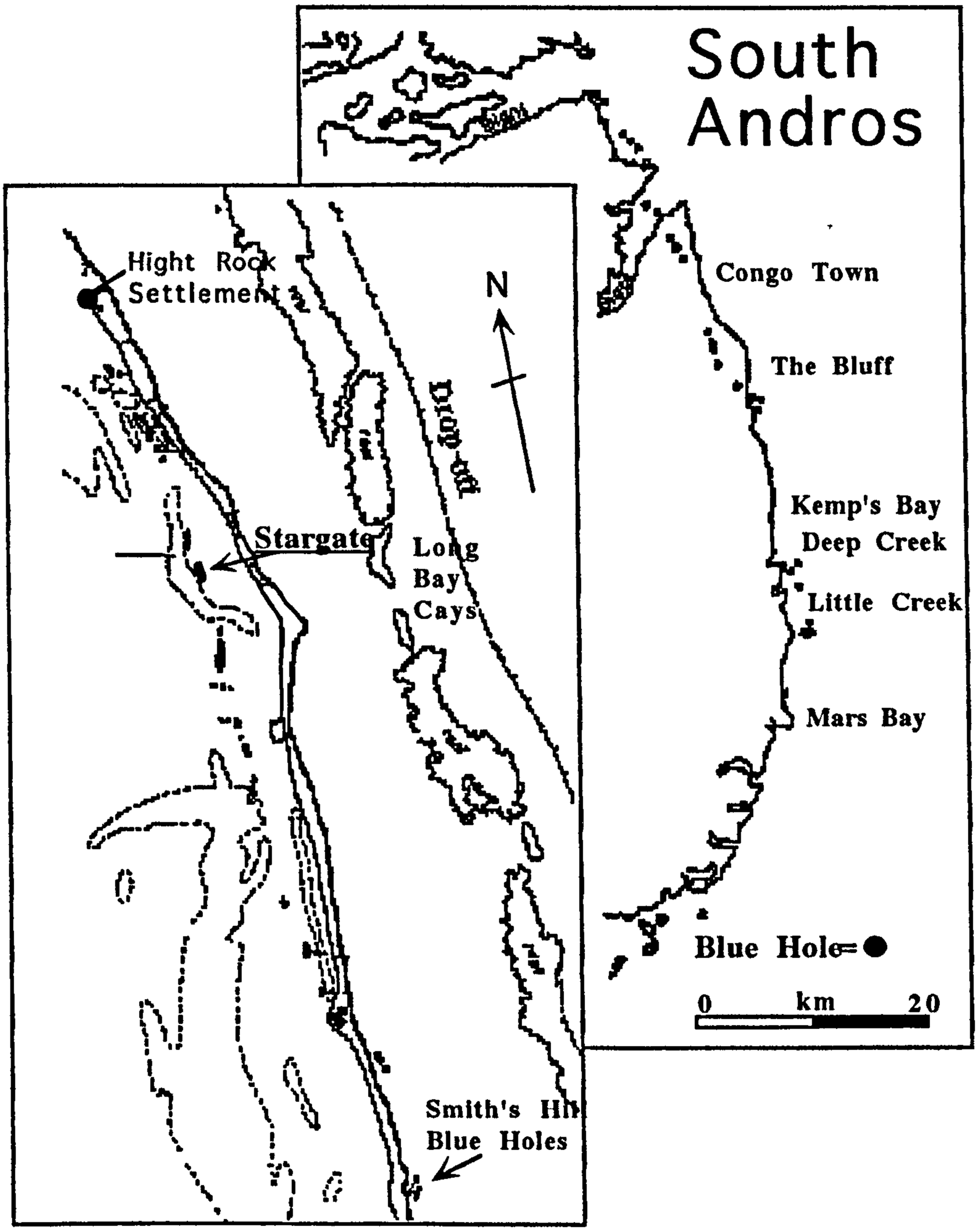


Figure 2.3: Map of South Andros showing location of Stargate Blue Hole.

The vertical depth directly below the entrance is 87 m with greater depths visible between the collapsed boulders which form the floor of the cave. To the north is a 4 to 5 m wide passage, with the roof at 28 m and the floor averaging between 60 and 75 m. This passage extends for 107 m to a boulder choke.

To the south, a passage similar in structure is entered through a more narrow constriction than to the north. The main south passage runs for 100 m to a further choke, passable on the right-hand side at 37 m to reach an extremely loose boulder chamber, choking again after further 30 m. This passage, however, has been extended through a deep unstable opening which opens up into another larger Stargate system.

This section of the cave has been named by Rob Palmer, who discovered it, "Dark Star". Back in the main passage, the entire floor of the cave is formed by massive boulders jammed across the rift, which have been planed from the walls by a combination of the effects of gravitational splitting during eustatic changes in sea level and microbial and chemical weakening of the wall rock. The average depth of this cave is about 75 m, whereas depths in excess of 100 m have been reported from other caves on the same fracture system.

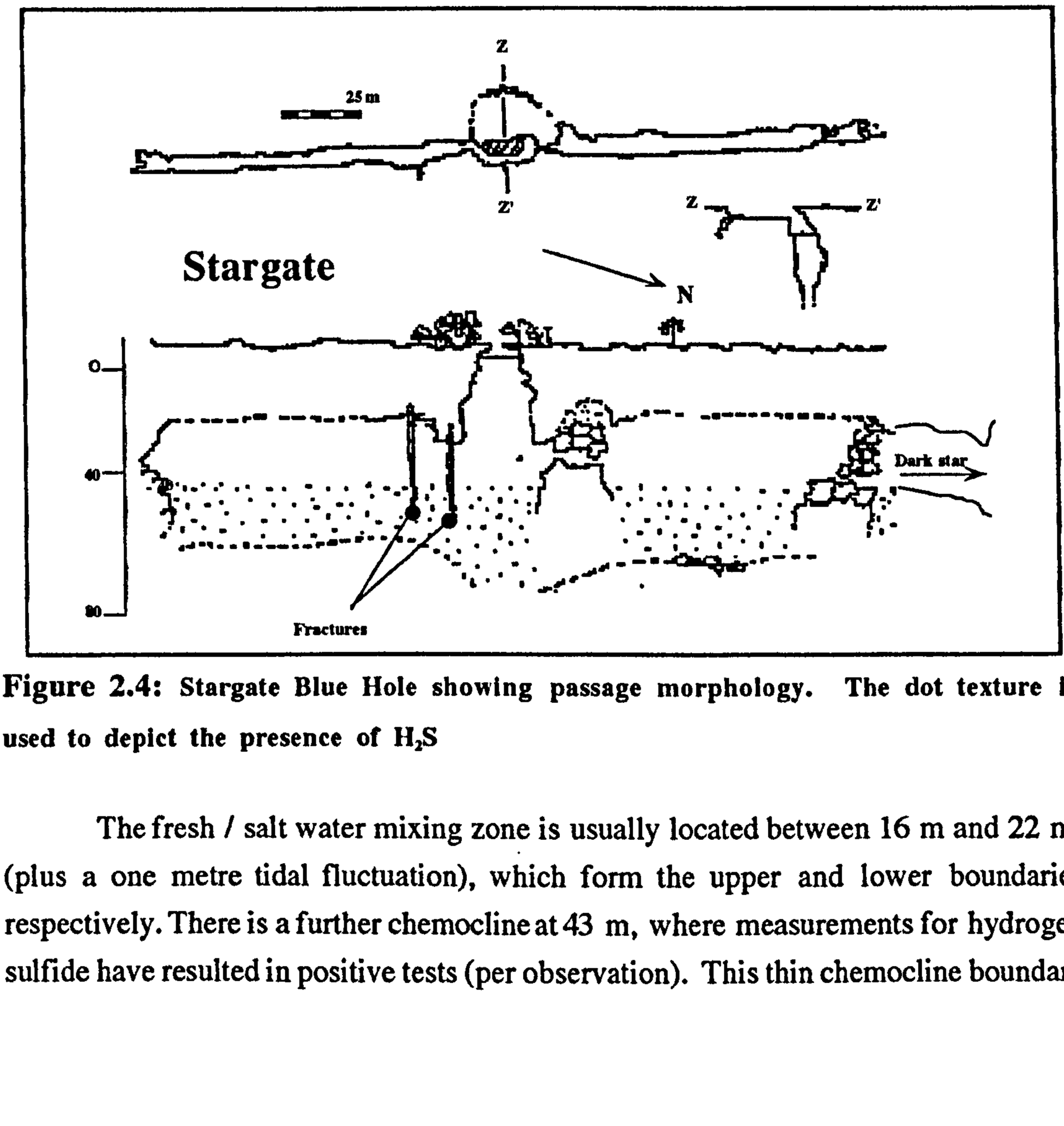


Figure 2.4: **Stargate Blue Hole showing passage morphology. The dot texture is used to depict the presence of H_2S**

The fresh / salt water mixing zone is usually located between 16 m and 22 m, (plus a one metre tidal fluctuation), which form the upper and lower boundaries respectively. There is a further chemocline at 43 m, where measurements for hydrogen sulfide have resulted in positive tests (per observation). This thin chemocline boundary

can also be observed as a thin gray line on the cave wall, and by a slight reduction in visibility within the water column as one passes through at this depth. Speleothems on the wall are evident at depths to 60 m (personal observation). The boulders of the cave floor are covered with a fine gray/brown sediment which is easily disturbed by divers.

3
Methods and Description of Methods

Introduction

Relatively simple and effective field techniques have been developed for the collection of water, rock and sediment samples for sterile bacterial work (Schwabe, and Wheeler, 1998). This was necessary because blue holes environments makes sampling difficult for several reasons: (1) some sites were highly decorated, forcing slow and exact movements; (2) off-loading equipment during sampling was not possible in most cases because cave floor sediments were thick and items could be lost; (3) air and depth limited time at sampling sites making collection sometimes very difficult. There is also the additional complication that if anything goes wrong underwater a simple escape to the surface was impossible from an underwater cave environment. Procedures needed to be both straightforward and efficient. Established cave diving techniques were used to access the sites (Prosser, and Grey, 1992) and oxygen enriched air was used to extend dive times beyond those allowed when using ordinary compressed air (Palmer, 1994). The sampling methods developed allowed improved collection procedures after the first field trip and more efficient use of in-water time.

Immediate oxygen, pH, temperature and alkalinity measurements were taken in the field while the rest of the work was completed back in the laboratory in Bristol, UK. Incubation of some water samples was completed by placing samples in sealed containers into the cave waters adjacent to the sample sites to ensure close to *in situ* conditions.

3:1 Field Sampling

Field sampling for this project was accomplished during two major field campaigns. The first in February 1994 to Lucayan Caverns and Owls Hole on Grand Bahama and the second in January and February 1996, to Stargate Blue Hole on South Andros and Lucayan Caverns Grand Bahama. Research results from both major field trips will be discussed separately because, although considerable refinement and improvement in sampling techniques occurred in the second field trip, the basic analytical approaches were very similar or identical and therefore the 2nd field trip allowed confirmation and extension of the preliminary results obtained in the 1st field

trip. The 1994 field trip was in part a reconnaissance trip, firstly to try out new field sampling methods and, secondly, to determine if bacteria were present and in what numbers. The second trip to Lucayan Caverns allowed for repeat sampling to compare with some of the 1994 results, but additional samples were also collected. The second half of the field trip focused on the Stargate Blue Hole, South Andros, where two weeks were spent collecting samples.

3:1:1 Water Sampling

The water column within Lucayan Caverns, Owl's Hole and Stargate Blue hole are highly stratified. Stratification layers have been found to be as thick as 4 or 5 cm or as thin as 1 or 2 cm. These layers are visual because they act as POM, a zone of living macrofauna (personal observation) traps. It is not known how quickly these minor layers, once disturbed, re-establish themselves, however, the major density interfaces are known to be back in place within 24 hours. Our objective was to sample these interfaces. Diving within these sites, however, disturbed and mixed the water column, making it necessary to use extensive numbers of sampling tubes (Figure 3.1) and once in place to leave the site to allow the stratified water column to re-establish itself.

In each cave system, a sampling site was chosen to fulfill the following criteria:

1) did not require a long or difficult dive access,
2) had no direct organic input from the surface, via cave openings,
3) allowed access to a full vertical water column through the mixing zone (fresh to marine water) determined by *in situ* conductivity measurements,
4) was large enough that, when the sample tubes were in place, the diver could be stationed far enough away from the sampling site so as not to disturb the stratified water column at the intake end of the tube.

Once such a site was selected for testing, measurements were taken using a WTW salinity/temperature meter in an underwater housing. These measurements were used to identify the depth of the fresh-saline transitions. To prepare the site for water sampling (Figure 3.1), one end of the line from a cave-reel was attached to the ceiling in the fresh water zone and the other end secured to the floor in the saline zone. Prior to installing the pre-cut silicon sampling tubes (I.D. 4.8 mm, supplier BDH, UK), the volume of the longest of the tubes was measured to determine the internal volume. The

tubes were then filled with filtered, sterile distilled water and capped at one end to prevent cave water from entering. The ends of several tubes were then attached to the line in the cave with nylon cable ties at pre-selected and measured depths; the other ends of the tubing (which were several metres long) were secured to a horizontal base line at the opposite side of the chamber. The caps on the intake side of the tube were removed prior to exiting the cave.

Prior to entering the cave, the water analysis instruments, i.e., dissolved oxygen, pH, salinity, temperature etc., (Figure 3.3) was set up and calibrated so that as divers were completing their in-water decompression, samples were placed into a plastic milk crate and lifted to the surface and placed into a cooler for immediate analysis by another member of the team. Calibration of the DO probe was done using a zero oxygen solution, 2 gm of Sodium Sulphite in 100 ml of water. The probe was allowed to polarize in the Sodium Sulphite solution before being removed. The probe was than calibrated for 100% by holding the probe 1 cm above a container of fresh water. Additional variations such as salinity and barometric pressure was calculated into the calibrations. Calibration of the pH probe was done before and after each batch of analyses with NBS high precision buffers (± 0.02 pH units). The salinity probe was calibrated using known Molar concentrations of KCL.

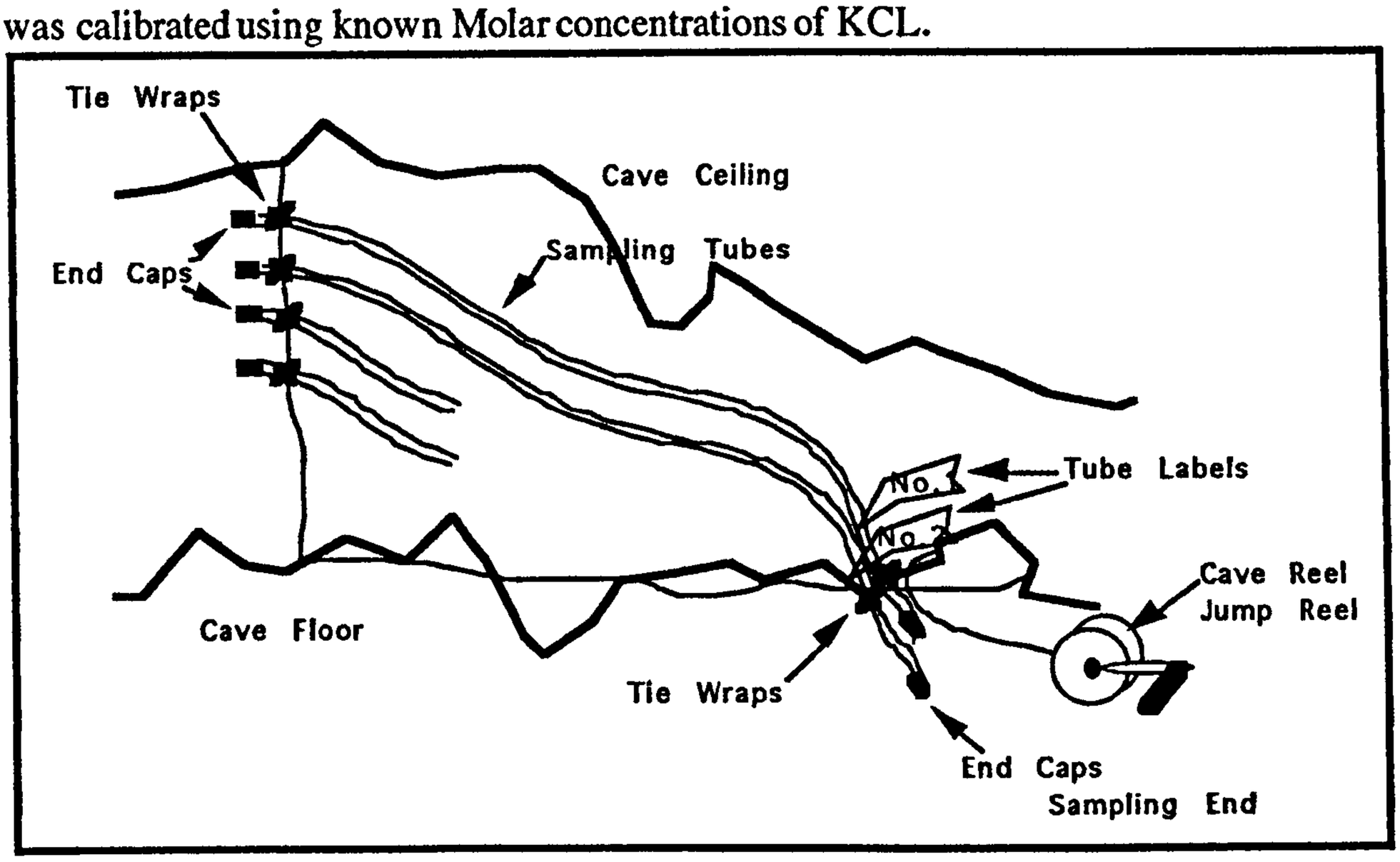


Figure 3.1: Water sampling method used to collect stratified water layers within the water column.

When it was time to collect the water samples, each tube was flushed with a cave water sample before sample collection by drawing measured amounts of water through the tube with a syringe and expelling it into the surrounding water. A separate syringe was used for each tube to avoid cross contamination. Water samples for

geochemical analysis were collected using 0.5 litre polyethylene water sample bags, medically known as "intravenous bags" (Figure 3.2). Intravenous bags were supplied by S.P. Services in Shropshire, UK. containing 0.5 litres of sterile 0.5% NaCl solution.

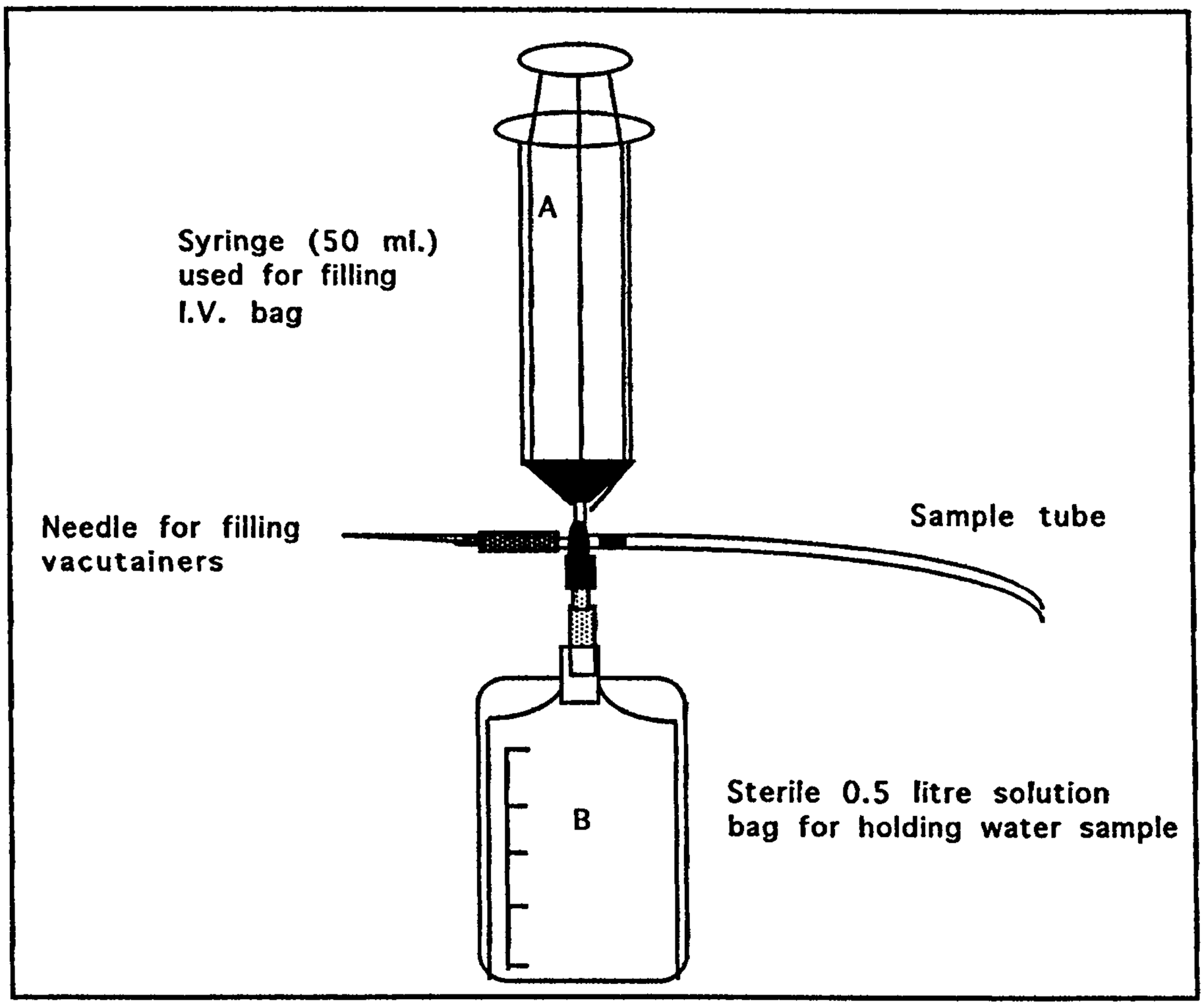


Figure 3.2: Sample collection system using intravenous bags and syringe

The saline solution was drawn out and the bags flushed four times with sterile distilled water to remove all remaining salt. The injection port of the bag was cut away and a sterile 3-way stopcock, (supplier Sherwood Medical), was attached. A sub-sample was removed and conductance was measured. Usually 100 ml of distilled water, flushed through empty bags three times, was enough to remove all residual salt solution. A further four flushes were conducted to ensure zero contamination. Following the flushing, all water and air was evacuated from the bag. A gas permeability test was performed on one bag prior to use in the cave. This test was to ensure that potentially anoxic water samples would not be contaminated by diffused oxygen from more oxygenated water or air during transport and sample handling. Water was boiled and cooled with filtered nitrogen to remove oxygen. This water was then reduced with a few drops of a 12% w/v sulfide solution, followed with a few drops of Resazurin (supplier Sigma Chemicals, UK), used as redox indicator. The bag

was then filled to capacity and left in air under ambient conditions. Approximately 3 hours later the water within the bag began to turn pink, indicating diffusion of oxygen into the bag. Hence, samples were immediately measured for oxygen content upon removal from the cave environment without storage as samples were usually processed within 15-30 minutes; any diffusion of oxygen into the sample would be minimal.

After flushing one of the intravenous bags was attached to the sample tube with a 3-way stopcock after the end caps were removed. Water was drawn up into a 50 ml syringe, (supplier Becton-Dickinson, Ireland) and, after changing the position of the 3-way stopcock, expelled into the bag. The syringe was removed and the bag was shaken and the water was sucked out again and expelled into the water surrounding the diver. This procedure was conducted three times for each bag to avoid any dilution by the distilled water. Once the flushing procedure was complete, the bag, the sample tube and the syringe all contained the water to be sampled and sample collection began. Water was drawn into the syringe, and ejected into the sample bag using the 3-way stop-cock. The bag was filled to capacity, approximately (600 ml) by repeating this maneuver, which took approximately 5 minutes per bag. Three bags were filled per sample depth: (1) one bag for geochemical analysis, (2) one for bacterial activity and (3) one for bacterial counts. The bags were then closed using the 3-way tap and placed in a BDH container for transport out of the cave. When all sampling was complete, the tubes were once again flushed with sterile distilled water which was carried in a spare bag and, if it was planned to use the tubes again at another depth or a different site, the tubes were once again flushed with sterile distilled water. The tubes were then capped at both ends using the blanking off caps which came with the stop-cocks. Although not all bags were dedicated to geochemical analysis, all three bags were measured for oxygen, pH, temperature and salinity. This was to ensure that the conditions surrounding the sample depth had not changed. Used bags were flushed with sterile distilled water, then flushed with 100 ml of 95% w/v ethanol, and flushed again three times with sterile distilled water. During the second field trip only 2 bags were needed and the samples for bacterial and biochemical tests, except for thymidine and acetate, were collected directly into vacutainers (refer to section 3:1:2).

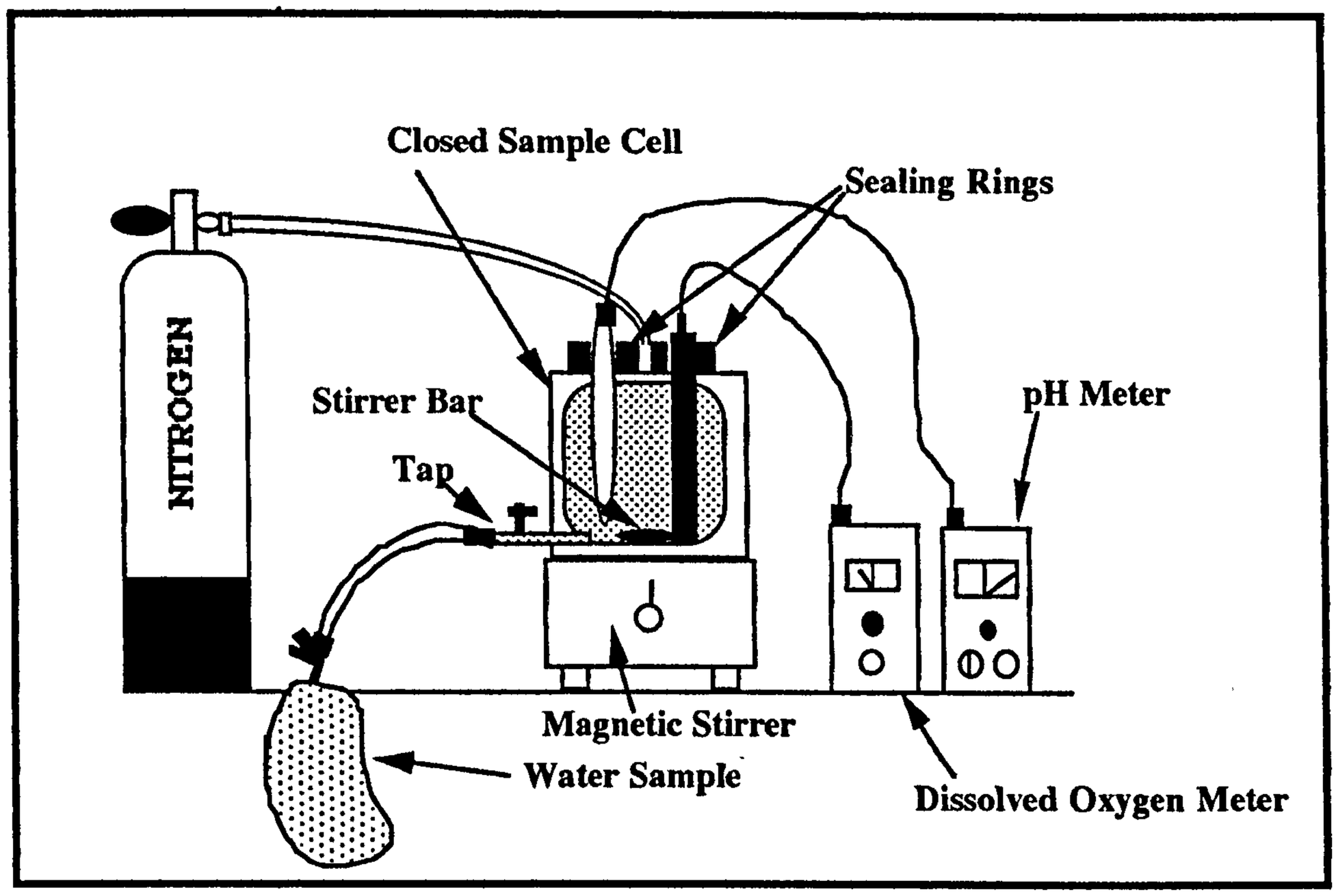


Figure 3.3: Field set-up for water analysis measuring dissolved oxygen, pH, temperature, and conductivity of water samples. The drain is where the tap is.

3:1:2 Vacutainers

Vacutainers were used to take samples for bacterial analysis on the second field trip as bacterial populations in the samples collected in Duran bottles were much higher than anticipated, enabling much smaller samples to be analysed. Water samples for bacterial counts and bacterial activity were collected using 10 ml tubes, BD (Becton Dickinson), sterile, no additive (Figure 3.4). Tubes were taped together in strips using duct tape, and individually labeled for different analyses or treatment. It is important to label both, tube and tape, because the label on the tubes came off once submerged in water.

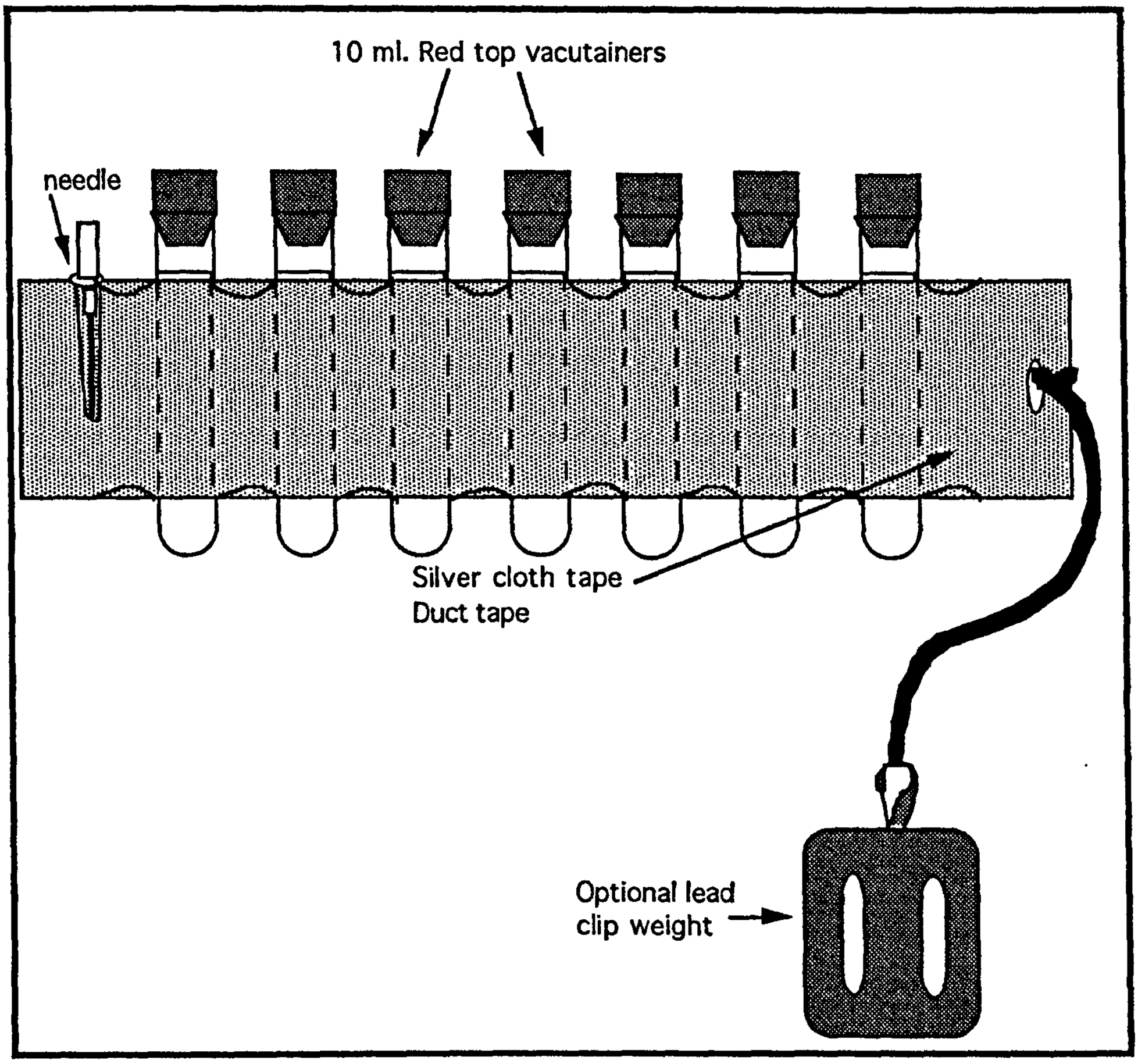


Figure 3.4: 10 ml sterile vacutainers used to collect water. The weight clip was necessary to prevent the buoyant tubes from floating away.

Taping the tubes together with the addition of a weight prevented them from being lost by buoyancy and allowed the tubes to float straight up in the water column. This made recognition of the differently labeled tubes and handling easier; entanglement was never a problem. When attaching the tubes with cloth tape (duct tape), it was imperative to leave at least 3 cm. of tube exposed at the red top end. This was to ensure, while underwater, that the diver could see where the water level was and not overfill the tubes. Head space must be left in the tubes or the red stoppers will blow off upon ascent.

These tubes were used primarily for Acridine Orange Direct Counts (AODC) sampling. The AODC-marked tubes contained approximately 1 ml. of 37% v/v formaldehyde which was injected into the tube prior to going into the field. Other tubes were used for (1) measuring bacterial activity using radioactive isotopes already added to the tubes prior to sampling, (2) particular geochemical analyses, such as hydrogen sulfide for which the tubes contained 20% (W/V) solution of zinc acetate.

Unfortunately, these tubes could not be used for metal and cation measurements, analyses which required acid as a preservative. Silicone coating used to prevent platelets from sticking to the inside of the tubes dissolved upon contact with acid. This was tested in the lab prior to field application.

Disposable 21 gauge needles, Sherwood Medical (Crawley, UK), were used for filling the vacutainers. These were taped alongside the tubes and upon removal inserted into the sample tube to allow direct fitting. Alternatively, the needles were attached to 50 ml BD syringes to inoculate the vacutainers. Doing it this way allowed more control as to how much water was collected in the vacutainers. The greater the depth of the sample, the greater the vacuum on the tubes. Use of double-ended needles further improved handling. Double-ended needles could be attached to the 3-way stop cock instead of the syringe.

3:1:3 Sediment Collection

Two types of sediments were collected, inorganic and organic. The inorganic sediment was recovered using "minicores", 28 cm long, made out of Plexiglas tubing (internal diameter 30 mm), which had one end chamfered to make insertion into the sediment easier. Rubber stoppers were used to close the ends of the cores, with the top stopper fitted with a 3-way stopcock, to allow release of water as the cores were pushed into the sediment. Prior to removing the core, the stop-cock was closed and as soon as the core was removed, the bottom stopper was replaced. In the lab back in the UK, core samples were placed on a cutting board and cut with a piece of sheet metal into 2mm sections, dried, and ground with mortar and pestle for XRD and SEM. Another core was cut in a sterile hood for bacterial examination. These samples were cut in 10 mm sections and put into sterile 30 ml serum vials with 1 ml of 4% v/v filter sterilized formaldehyde. The organic sediment collected for this study was soft and flocculent and could be measured to depths in excess of 1 metre on the floor of the open passage ways. This material was very difficult to collect because it was easily disturbed by any water movement generated by the diver. The moment the diver came too close to the accumulated layers on the cave floor, or gave a misplaced fin stroke, re-suspension of this material into the water column would occur, requiring several days for it to settle back out onto the cave floor. Similar organic floc material adhered to the cave ceiling and walls and was easily knocked into the water column by exhaust bubbles from open circuit divers. Samples were collected from the floor, walls and ceiling using different methods. Organic layers on the ceiling and walls was collected by sucking it off the wall using a 100 ml sterile syringe. The sample was kept in the syringe during the

course of the dive. Once outside the cave, 10 ml of samples was injected into sterile 25 ml glass serum vials which contained 1 ml of filter-sterililized 4% V/V solution of formaldehyde. The bottles were sealed using a sterile septa and aluminum crimp. The organic flocculent material from the cave floor was collected using sterile 100 ml syringes. The samples (10 ml) again remained in the syringe until they were brought out of the cave. These samples were also stored in 25 ml sterile serum vials containing 1 ml of 4% V/V solution of formaldehyde.

3:1:4 Special Techniques

- **Collection of Cave Rock**

Rock core collection was made possible by the use of a modified commercial impact air wrench, Clarke air model number CAT 23B (Figure 3.5), purchased from a local hardware store in Bristol, UK. The existing metal components within the unit were composed of iron and aluminum and were found not to be compatible with seawater. All moving metal parts were thus replaced with stainless steel. Being open to the water lubricants were not needed for the air wrench,. This was a bonus since we did not wish to contaminate the cave environment with any hydrocarbon-based oils. After each use, however, the drill had to be opened, washed out with fresh water and dried thoroughly because, despite the fact that the internal components were made of stainless steel, the aluminum drill housing would still oxidize and interfere with smooth operation.

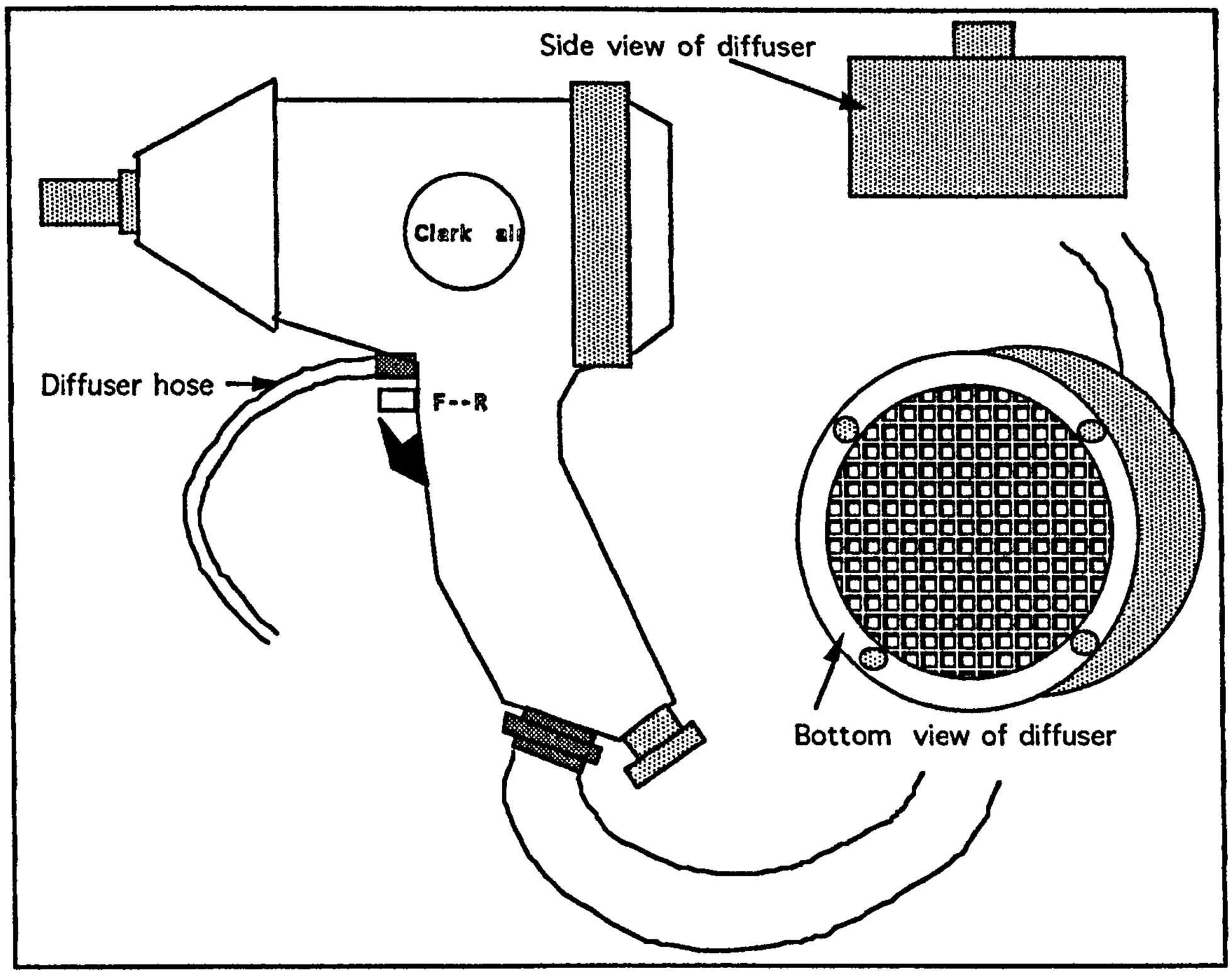


Figure 3.5: Air drill used for collecting rock cores.

The air hose for the drill was fitted with an adapter which would allow the hose to be connected to the first stage of a scuba regulator. This permitted the use of a standard 12 to 15 litre scuba cylinder as the air source for driving the air wrench. Another hose with a dispersion unit was attached to the exhaust port of the air wrench which floated above the operator, well out of the way of the sampling site area and thus preventing exhaust gases to disturb flocculent sediments and wall coatings and thereby reduce visibility. The dispersion unit was built from a piece of PVC pipe approximately 8 cm in diameter and 4 cm deep. One end was sealed with a fitting to accommodate the exhaust hose, while the removable exhaust end had a plastic screen. Finally, a large pore sponge was inserted into the unit. Despite the use of the dispersion unit, some disturbance was inevitable and it is for this reason that drilling was the last project worked on in the cave (especially since particulate organic matter (POM), total organic carbon (TOC), and dissolved organic carbon (DOC) was being measured).

The diamond-tipped corer itself was built into a PVC base designed by Fred Wheeler of the University of Bristol, Geology Department. The base was built with three independently movable stainless steel pins (Figure 3.6). and controlled by a cam located on the corer base. When the base was placed against the wall, the cam was

released and this independent movement of the pins allowed setting of the corer perpendicular to the wall, irrespective of irregularities. Once a site was chosen, the pins were locked into position.

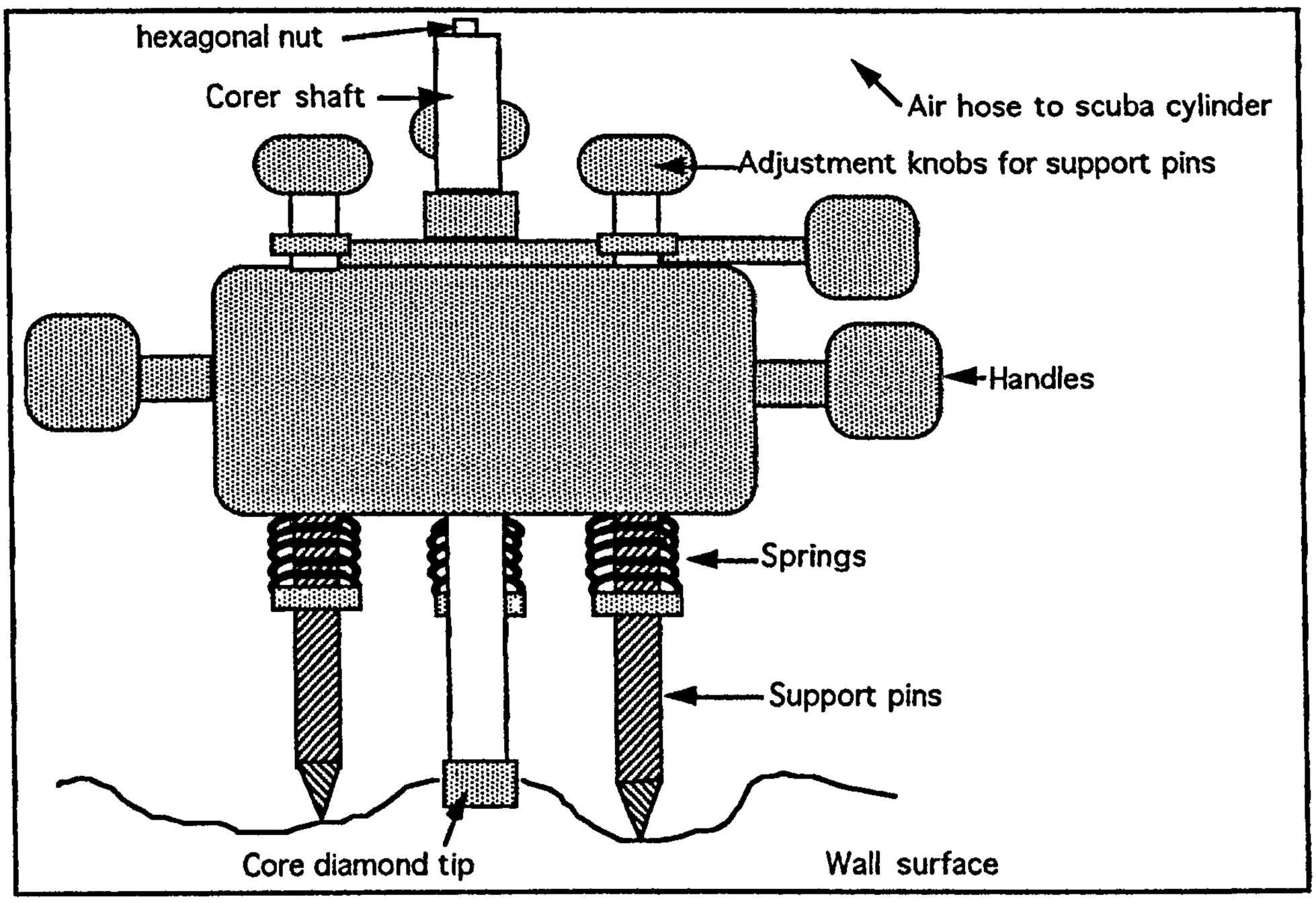


Figure 3.6: Drilling base used together with the rock drill.

The corer shaft (which was custom made by Durbin Metal Industries in Bowling Hill, UK) was set into the centre of the base, allowing some free up-and-down movement. The corer shaft itself was fitted with a male luer hexagonal nut which coupled with the female luer hexagonal nut of the pneumatic wrench. Once the base was in place, the air wrench was placed on the corer tip and drilling started. Only minimum pressure was needed during coring, in fact, the less pressure used, the better the corer performed.

To remove the rock core from the wall, the drill base was removed and a stainless steel tube approximately 10 cm long, which fitted the rock core exactly, was slipped over the core end and with a gentle tap on the side of the tube the core was released from the wall. The rock cores were stored for transportation in 30 ml, sterile, clear polypropylene tubes. A sterilized polypropylene sponge, (supplier BDH) was inserted on top of the core. This was done to prevent movement of the core within the container and the possible loss of true *in situ* wall orientation of the core.

3:2: Biochemistry and Bacterial Techniques

3:2:1 Acetate Measurements

Acetate is an important substrate for heterotrophic bacteria, especially in anoxic and low dissolved oxygen environments, environments thought to be present in the Bahamian cave systems. Acetate is a key intermediate in the processes of degradation of organic substances in sedimentary environments (Wellsbury and Parkes, 1995); it is the primary substrate for sulphate reduction in marine sediments (Parkes et al., 1989; Sørensen et al., 1981) and methane production in fresh water sediments (Cappenberg and Prins, 1974) and in anaerobic digestion (Colleran, 1992). Volatile fatty acids (VFA) are very important, both as the major end-product of fermentation and as substrates for terminal carbon mineralization in sediments. The key intermediate in the processes controlling the degradation and preservation of organic matter in sedimentary environments is acetate (Wellsbury and Parkes, 1995).

To accurately determine acetate turnover rates from radio-labeled turnover rates of ^{14}C acetate, the *in situ* acetate concentrations need to be measured. Bioavailable acetate is analyzed by using high pressure liquid chromatography techniques (HPLC); based on the reaction of acetate with acetyl coenzyme. Two methods of acetate determination were used; a) bioavailable, only used at the Stargate site and b) total, used on the 1994 field trip. Total acetate concentration was analyzed after derivatisation using a modified 2NPH procedure (Wellsbury and Parkes, 1995; Parkes et al, 1989; Mueller and Parkes, 1987; Miwa and Yamamoto, 1985; Miwa et al, 1980). One ml of a previously filtered and frozen sample was transferred into a 4 ml chromacol vial (Phase Separations Ltd. organic free (heated to 450°C for 4 hours prior to use). 0.4 ml of EDAC solution (0.25 M 1-ethyl-3-{3-dimethylaminopropl}-carbodiimide hydrochloride in a 3% (v/v) solution of pyridine in ethanol) was added immediately followed by 0.2 ml of 2NPH solution (0.02 M 2-nitrophenylhydrazine hydrochloride in water). The contents of the vial were mixed thoroughly and incubated for 1 hour at 25°C. Following the incubation period, 0.1 ml of KOH solution (1.5% (w/v) in 80% (v/v) methanol: water solution) was added, followed by mixing and heating to 60°C for 15 minutes. The samples were then cooled to room temperature, followed by adding 0.1 ml of 3 M HCL. After mixing for 5 minutes 2.0 ml of HPLC grade chloroform was added. Following thorough mixing and separation, the lower chloroform phase was removed by suction to a clean chromacol vial. The chloroform was then evaporated to dryness at 60°C prior to re-suspension of the derivatised sample in 400 μl of 25% (v/v) acetonitrile: water solution. This was then filtered, syringe-(0.45 μm,

Waters LTD) and injected into the HPLC via an auto-sampler (Waters Ltd.). 10 μl sample injections, kept at 10°C, were layed onto a reverse phase C^{18} cartridge column (Waters Nova-Pak C18 cartridge, 3.9 x 150 mm, 60 Å, 4μm packing) at 45°C. Gradient elution was used to optimize peak separation with UV/vis detection at 400 nm and peak area integration used for quantification . After each sample run a 15 minute stabilization period was maintained before the injection of the next sample. A synthase (King, 1991), according to the following stoichiometry is:

$$\text{acetate} + \text{ATP} + \text{CoA} \xrightarrow{\text{acetyl CoA synthase}} \text{X acetyl CoA} + \text{AMP} + PP_i$$

AMP production is measured because it is directly proportionate to the amount of acetate originally present, and as this is an enzymatic reaction, it measures the bio-available acetate pool concentration (Wellsbury and Parkes, 1995).

3:2:2 Analysis of Acetate and Bicarbonate Activity Measurement

Water column samples were used to inoculate 30 ml sealed sterile serum vials with (Sigma Chemical) 2 μCi (2.9 x 10^{-6} DPM) of ^{14}C bicarbonate and 2μCi (2.2 x 10^{-6} DPM) ^{14}C acetate. Parallel samples were incubated for both the bicarbonate (2 and 5 days) and the acetate (2 and 6 hour). The serum vials were placed inside sealed bags, placed in sealed ammunition boxes, and allowed to incubate in cave water, well away from the entrance. To terminate incubations, both the bicarbonate and acetate samples where injected with 1 ml of 4 M NaOH at the appropriate times.

[^{14} C] acetate was measured using a modified version of the method described by Wellsbury and Parkes, 1995. The 30 ml serum vials were opened and a small magnetic stirrer was dropped into each vial. Sealed again, samples were secured on top of a magnetic stirrer and a large gas inlet needle was pierced through the septa. Three scintillation vials containing 10 ml of CO_2-absorbing scintillate were used. The scintillation cocktail was made up from (1600 ml toluene, 160 ml of methanol; 140 ml Beta-phenylethylamine, 10.0 g of 2,5-Diphenyloxazole 99% scintillation grade PPO; 0.2 g (1,4-bis[5-Phenyl-2-oxazolyl]benzene; 2.2'-p-Phenylene-bi[5-phenylozxazolel] (POPOP)); this makes approximately 2 litres. The sample was then acidified with 2.0 ml of 2M H_2SO4 and sparged for 60 minutes. with oxygen free nitrogen (OFN) at a flow rate of 35 ml min-1.

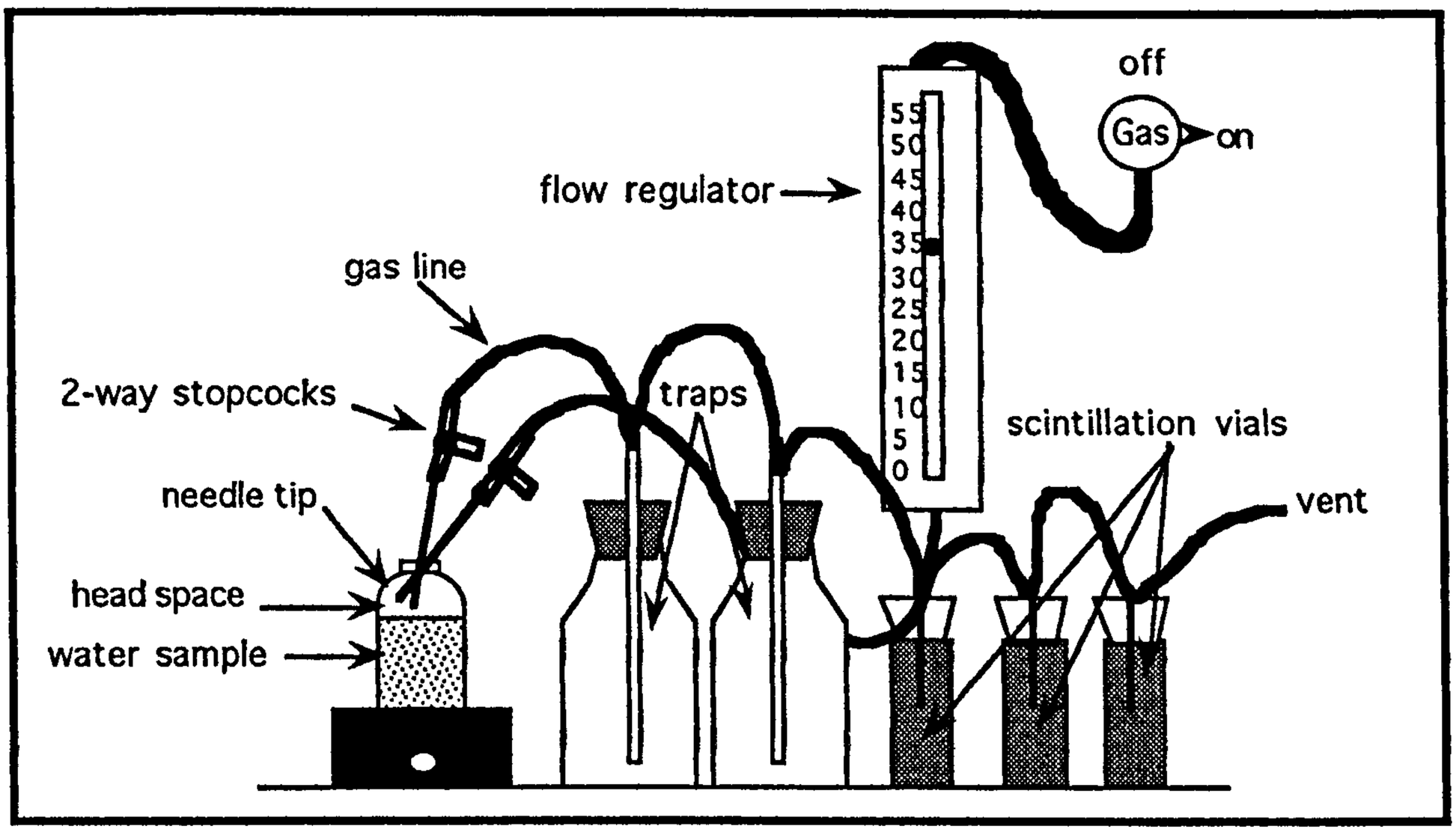


Figure 3.7: ^{14}C Flush system used for radio-labeled carbon recovery. This system was used for both ^{14}C bicarbonate and ^{14}C acetate tracers.

Two empty traps (50 ml) were positioned in the system between sample vial and scintillation vial to prevent physical carry-over of label as aerosols following injection of acid. The scintillation vials were removed for liquid scintillation counting (Pharmacia-LKB 1410). Between sample elutions the system was flushed for 10 min. with OFN at 100 ml min-1. To measure residual [^{14}C] acetate, 5 ml of the acidified porewater were added to 5 ml of Instagel (Packard Instruments) in triplicate, mixed thoroughly and counted as above. Trapping efficiency of $^{14}CO_2$ using this system was always >96%.

The ^{14}C bicarbonate samples were filtered through a 0.2 μm filter. Samples were washed 3 times with 5 ml of distilled water. Filters were dried in an oven at 37°C for 1 hour before placing each filter into a scintillation vial containing 5 ml Instagel (Packard Instruments) to be counted (Pharmacia-LKB 1410 liquid scintillation counter).

3:2:3 Incorporation of Thymidine into Bacterial DNA

Water samples for thymidine incorporation measurements were collected in intravenous bags (Figure 3.2). In the field lab, 10 μl (10 μCi) of [methyl-^{3}H] thymidine solution (83 Ci mmol^{-1}, Amersham International), was injected into 10 ml red-top vacutainers (Figure 3.4). Prior to the needle being pushed into the stock vial containing the [methyl- ^{3}H] thymidine solution, the needle was flamed over a gas

burner to prevent any possible contamination to the stock or subsequent sample vials. Following the label injections, 5 ml of water sample was injected into the vacutainers and the time marked. These samples were incubated for 15 minutes prior to termination of incubations by injecting into the sample vial 5 ml of 5%, v/v trichloroacetic acid (TCA) at 4^0C on ice. Blanks were prepared by addition of 5 ml of TCA to a vial containing isotope only.

Short incubation periods, as described above, are crucial (Moriarty, 1990) because long-term incubations are prone to errors arising from thymidine catabolism, resulting in labeling of ^{3}H-DNA via routes other than the thymidine salvage pathway. Another problem which is minimized by short incubation times is that introduction of the label may in itself constitute the introduction of a growth substrate and thereby promote cell growth (Moriarty, 1990).

Macromolecular material precipitated from cells lysed in the 5% w/v TCA on ice (at 4°C), was filtered under vacuum through a 0.20 μm filter (Figure 3.7). TCA in solution material on the filter was rinsed three times with 3ml of cold 5% TCA solution and twice with 95%, cold ethanol. All rinses were collected, and the ^{3}H activity determined by liquid scintillation counting. The rinsed filter was dried under vacuum, transferred into a centrifuge tube and hydrolyzed in 1M NaOH for 50 minutes at 37°C in an incubator to solubilize the nucleic acids. Centrifugation separated protein, found in the pellet, from soluble RNA and DNA in the supernatant.

DNA was re-precipitated from the hydrolytic supernatant with 20%w/v TCA in 3.6M HCl (to acidify the alkaline hydrolysis solution) at 4°C. "Cold" carrier DNA (50 μl of a saturated solution) and Kieselguhr were added to the cooled acidified solution to aid precipitation and cooling. Once cooled, the sample was centrifuged and 5 ml of the supernatant was added to Instagel for measuring the radiolabelled RNA fraction. The Kieselguhr/DNA pellet was rinsed with acid and alcohol to remove any remaining soluble radiolabel; DNA was extracted from the precipitate in 5% w/v TCA at 100°C for 30 minutes, and ^{3}H-DNA activity determined by liquid scintillation counting. The distribution of ^{3}H in protein (hydrolyzed from the original filter following a second hydrolysis for 18 hours at the increased concentration of 2M NaOH, incubated at 37°C) was determined.

Bacterial productivity (mgC m^{-2} d^{-1}) was calculated from the rate of thymidine incorporation converted to bacterial cells, (conversion factor of 2×10^{18} cells mol^{-1} thymidine incorporated, (Moriarty, 1986), and then to bacterial carbon (conversion factor of 310 fgC μm^{-3}, Fry, 1990).

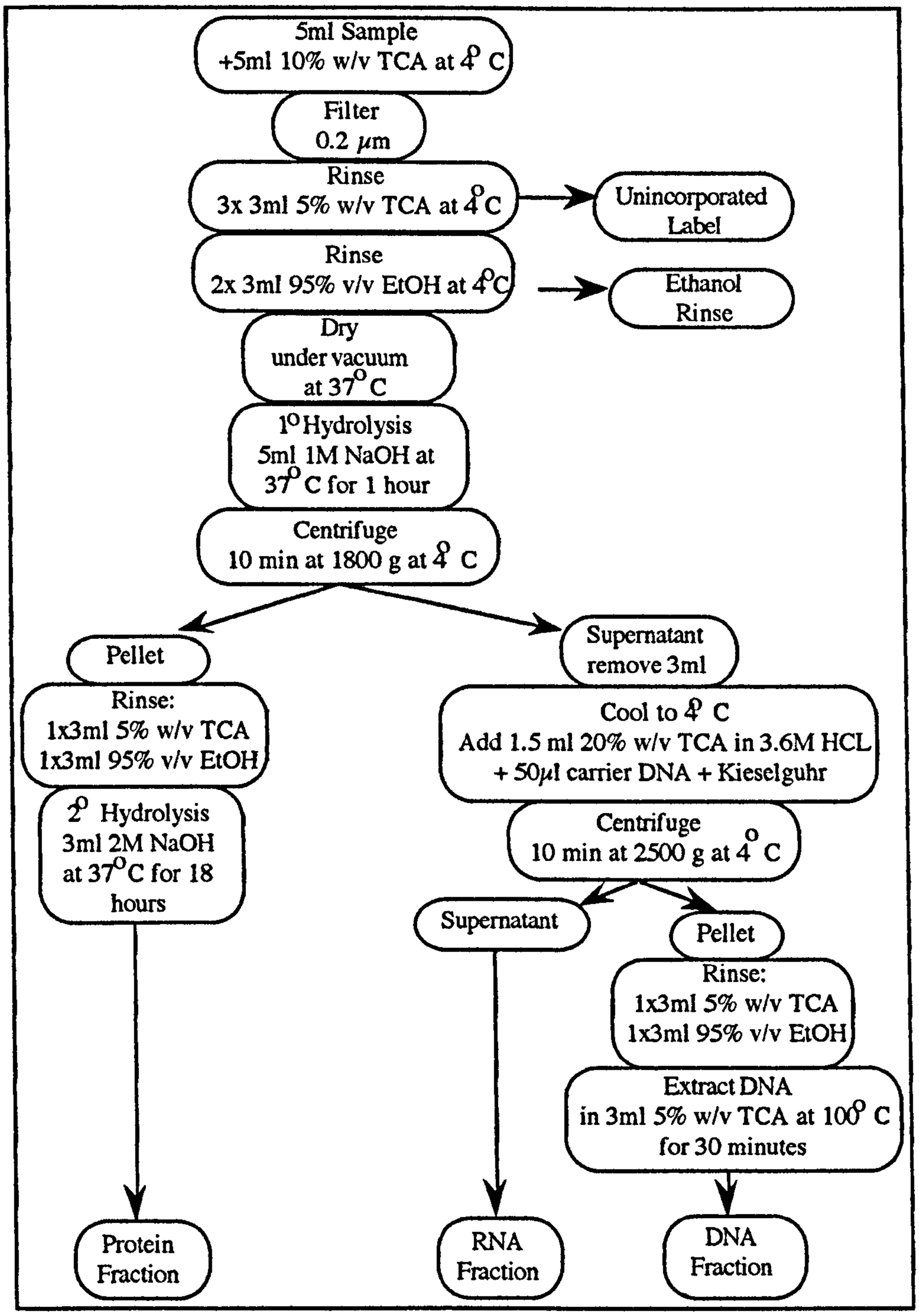


Figure 3.8: Flow chart showing the step-by-step procedure for fractionation/extraction protocol for thymidine incorporation rate measurements in water samples containing bacteria (re-drawn from Wellsbury, 1992)

3:2:4 Sulphate Reduction Rates

Rates of bacterial sulphate reduction were measured from production of ^{35}S-sulfide in water samples incubated with $^{35}SO_4^{2-}$ (Jørgensen, 1978; Fossing et al;

1989). Water column samples were used to inoculate 30 ml sealed sterile serum vials with Sigma Chemical, 2 μCi (4.4 x 10^{-6}DPM) of ^{35}S Sulphate. Parallel samples were incubated for 2 days and 5 days. The incubations were stopped by the addition of 4 ml of 20% zinc acetate (w/v). Samples were later distilled for total reduced inorganic sulfides (TRIS) by a single-step chromium-reduction method (Fossing et al; 1989) on a special glass rig which enables distillation of six samples concurrently (Parkes and Buckingham, 1986). A 10 ml aliquot of the stored sample was transferred to a 125 ml BDH distillation flask containing a small stirrer . Exactly 5 ml of a 20% NaCl (w/v) was added in addition to the water to increase the volume after which 5 ml of absolute ethanol was added. The flasks were attached to a distillation apparatus and flushed for 20 minutes (flow rate 50 ml / min.) with oxygen free nitrogen (OFN). After flushing, 25 ml of reduced chromous chloride (1M solution in 0.5 M HCl, reduced in a zinc amalgam column) was added by injection through a side-port septum, along with 10 ml of 11.6 M HCl. Generally two vials at a time would receive CrCl, followed quickly by the concentrated HCl. Samples were left stirring for 45 minutes on hot plates set to 80°C (heated magnetic stirrer, Stuart Scientific). ^{35}S sulphide was collected in a 25 ml glass trap containing 10% (w/v) zinc acetate.

A second trap containing a 10% (w/v) solution of cadmium chloride was attached to the exhaust from the zinc acetate trap as a visual check for complete removal of sulphide in the zinc acetate traps (sulphide carry-over would be a yellow cadmium sulphide solution). This trap remained colorless throughout all distillations reflecting the low S^{2-} concentration of the sample. After 45 minutes, the distillation traps were removed, vortexed for approximately 15 seconds, and 5ml added to 5ml of "Instagel 2" scintillation fluid and measured on a liquid scintillation counter to measure ^{35}S-sulphide (A) (Pharmacia-LKB 1410 liquid scintillation counter). The residual sample in the distillation flask was diluted to 100 ml and 5 ml of sample was removed and added to 5 ml of "Instagel 2" scintillation fluid and counted as previously described, this measured residual ^{35}S-$SO_4$2- (B). Rates of bacterial sulphate reduction were calculated as described by Jørgensen, 1978.

$$Rate = \frac{[SO_4^{2-}].(^{35}S - H_2S).\alpha}{(^{35}S - SO_4^{2-}).t}$$

where $[SO_4^{2-}]$ is the sulphate pool size in nmol ml/day

- $(^{35}S\text{-}H_2S)$ is the total sulfide radioactivity (**A**)
- $(^{35}S\text{-}SO_4^{2-})$ is the total injected radioactivity of sulphate (**B**)
- **t** = is the incubation period in days;
- and α is the isotope fractionation factor (=1.06)

A 1 ml volume was removed from the zinc acetate trap for determination of total distilled sulfide by spectrophotometry.

3:2:5 Sulphide

Water column samples, stored in 4% w/v zinc acetate solution, were analyzed according to a modified Cline (1969) procedure (Parkes and Buckingham, 1986). 1 ml aliquots of sample were analysed, using 1ml of mixed diamine sulphate/ferric chloride reagent (in 50% v/v Hcl). The sample was mixed thoroughly and incubated for 20 minutes prior to dilution to 250 ml with distilled water. Absorbance at 670 nm was measured (Cecil CE292, Cecil Instruments, Cambridge) against a series of calibration standards (0, 0.05, 0.1, 0.15, 0.2 and 0.25 mgS), and corrected for dilution by zinc acetate.

3:2:6 Epifluorescence Microscopy

Water samples during field trip 1 were collected in 500 ml Duran bottles and stored with filter-sterilized (0.2 μm pore size, Nucleopore) 4% (v/v) formaldehyde. On the 2nd field trip, the collection of water samples was modified by using 10 ml vacutainers (refer to figure 3.4). All glassware was heated to 450°C for 1 hour to remove any organic residue. Volumes of water samples, taken for subsequent analysis, were varied to produce an acceptable number of cells per field of view. Volumes ranged from 1 ml to 10 ml. Samples were removed with a sterile Gilson pipette tip and added to a sterile disposable 'Sterilin' bottle (Merck Ltd.), containing 10 ml of membrane-filtered 2% (v/v) formaldehyde solution. A 10 μl volume of filter-sterilized Acridine orange solution was added (stock solution 5 g/l containing 4% (v/v) formaldehyde,

kept refrigerated). The sample was then vortexed, mixed, and left for 5 minutes at room temperature. The sample was again vortexed and vacuum-filtered through a black 0.2 μm nucleopore membrane. The black membranes are used to suppress auto fluorescence under excitation (Fry, 1988) and provide contrast for stained bacterial cells. The membrane was then mounted onto a glass slide using liquid paraffin, and examined under incident epifluorescent illumination (50W mercury vapor lamp) using a Zeiss Axioscop microscope and a 100x objective (Plan Neofluor oil-immersion and 10x eyepiece).

Total cell counts (Acridine Orange Direct Count, AODC), and the number of divided and dividing cells (Hagstöm et al, 1979; Getliff, 1991) were determined. Bacteria were counted in a 3 x 3 square grid (eyepiece graticule) until a count of 400 cells was reached, or 100 fields were viewed. Cells were described as being attached or free. The number of bacteria on particles was doubled to account for probable masking (Goulder, 1977) such that:

$$\text{Total cells} = (2 \times \text{“on particles”}) + \text{“off particles”}$$

Dividing cells, showing a clear invagination of the cell wall, are counted as one cell in both the total and dividing/divided cell count. Divided cells appear as adjacent cells with identical morphologies, and are counted as two cells in both the total cell count and the dividing and divided cell count (Getliff, 1991). The basic equation for calculating AODC [3.1]

$$\frac{\text{Mean number of cells per field of view } (\bar{x}) \times \text{Effective area of filter } (\mu m^2)}{\text{Area of field of view } (\mu m) \times \text{Volume of sample } (\mu m^{-1})} = \text{cells } \mu l^{-1} \qquad [3.1]$$

3:2:7 Viable Bacterial population (First Field Trip Only)

Viable bacterial populations were determined by filtering known volumes of cave water through a sterile 0.1 μm Millipore filter membrane and placing it on sterile agar plates containing specific media for different bacteria types and with different salinities (marine being 100% marine, brackish being half marine and fresh containing no marine water). Three types of media were prepared; Postgate's (1) sulphate reducing bacteria (SRB) (Postgate, 1984), (2) heterotrophic nutrient agar media, and a media (3) for Thiobacillus (obligate chemolithotroph) (Table 3.1).

Table 3.1: Preparation and handling of media used for bacterial culture experiments.

Media type	Fresh water	Salinity Brackish	Salinity Marine	anoxic	oxic
Sulphate-reducing	X	X	X	X	
Heterotrophic				X	X
Litho autotrophic	X	X	X		X

The media prepared for heterotrophic bacteria consisted of:

nutrient broth (13g/litre)

solution of agar (2% w/v) 2g/100 ml

Half of the plates were prepared anaerobically in a anaerobic cabinet for potential anaerobic growth, and the other half aerobically in a laminar flow cabinet to accommodate aerobic growth within water samples.

Postgate's media was prepared with varying salt concentrations to accommodate the varying salt concentrations within the water column, (i.e., fresh, brackish, marine).

Anaerobic Solution (A) Add respective amounts of NaCl and MgCl

K_2HPO_4 (0.5 g)

NH_4Cl (1.0 g)

Na_2SO_4 (1.0 g)

$CaCl_2 \times 2H_2O$ (0.1 g)

$MgSO_4 \times 7 H_2O$ (2.0 g)

Yeast extract (1.0 g)

Distilled water (980.0 ml)

Solution (B)

$FeSO_4 \times 7H2O$ (0.5 g)

Distilled water (10.0 ml)

Solution (C)

Sodium thioglycolate (0.1.g)

Ascorbic acid (0.1 g)

Distilled water (10.0 ml)

Filter (sterile) solution (**C**) and store in sterile vial. Heat solution (**A**) to a boil for a few minutes, then cool to room temperature while gassing with O_2 free N_2 gas (OFN). Add solution (**B**) and adjust pH to 7.8 with 1M NaOH solution. Autoclave with Agar (1 litre= 20g Agar) in a flask with non-absorbent cotton wool plug to prevent subsequent contamination. Remove from the autoclave after 20 minutes and let cool until one can hold the bottle for 30 seconds, then add solution (**C**). Place in an anaerobic cabinet (Forma Scientfic). Just prior to solidification the media is poured into petri dishes, (placed in anaerobic cabinet over night to remove oxygen from the plastic) while continuously swirling the remaining media in the flask, until all plates are poured. The swirling is done to distribute the grey precipitate suspended in the media.

The aerobic media for obligate chemolithotrophs was also prepared with varying salt concentrations and all plates that were prepared for travel to the Bahamas were stored in gas tight distribate aluminium bags (Cragg et al., 1992) with anaerocult (Merck) oxygen scrubber. Only plates which were aerobic media were sealed in aluminum bags without anaerocults.

aerobic medium (per litre)
KNO_3 (2.0 g)
NH_4CL (1.0 g)
KH_2PO_4 (2.0 g)
$NaHCO_3$ (2.0 g)
$MgSO_4$ *7 H_2O (0.8 g)
$Na_2S_2O_3$ * 5 H_2O (1.0 ml)
(pH 6.8-7.0)
Agar
The media was autoclaved in a flask with N/A cotton wool, allowed to cool, then plates were poured in laminar flow cabinet. Once the plates were cooled, they were turned over and allowed to dry. This is to prevent water condensation from dripping onto the surface of the media.

In the Bahamas we created a temporary lab which was set up in an abandoned army base barracks. A room was chosen which was almost completely tiled, for the reason that it was easy to clean. The whole room was wiped down with bleach and all windows and other opening were covered with plastic sheeting to insure minimal air flow which could potentially be carrying dust particles. Plastic sheet was erected over a stainless steel table and filtered N_2 was used to keep the tent anaerobic. An oxygen metre was set inside the hood to monitor O_2 levels. Anaerobic plates were open in the safety of the hood and aerobic plates were open and used in the confines of the tiled room.

From each sample specific volumes of water was filtered through a sterile 0.1 μM filter paper marked with grid lines for easy counting. The filter paper was then placed face down on the media. Anaerobic samples were placed back into the aluminum bags with Anaerocults and heat sealed and the aerobic samples were placed inside black plastic bags and the open end left draped down to prevent light from entering the bag but allowing air in. Plates were examined for bacterial growth within 24 hours after innoculation.

3:2:8 Amino acid analysis

Amino acids are the monomeric units of proteins containing carbon, hydrogen, oxygen, nitrogen, and for two monomeric units, sulfur and one selenium (Jones, 1994). The chemical properties of amino acids are, to a major degree, governed by the nature of the side chain, allowing amino acids that show similar chemical properties to be grouped into amino acid "families". These families are ionizable acidic, ionizable basic, non-ionizable polar, and nonpolar (hydrophobic) (Jones, J., 1994).

The flocculent mung was divided into two samples on the basis of pigmentation. One sample was dark brown, the other pale yellow. The samples were washed with distilled water, centrifuged and the pellets taken up in 6N.HCl, transferred to a hydrolysis vial and hydrolyzed at 117° for 16 hours under nitrogen. The hydrolyzed samples were taken to dry ice, dissolved in a neutralizing solution and dried again. The bilateral amino acids where than derivatised with phenylisuthiocyanate in preparation for separation on a reversed phase C^{18} column. Amino acids were detected as they emerged from the column by UV absorbence at 259 nm. A sample of the supernatant of the end of the two mung samples where run through the analyzer and hydrolyzed in order to detect for amino acids.

3:3 Geochemical Techniques

3:3:1 Dissolved Organic Carbon and Particulate Organic Matter

The Shimadzu total organic carbon analyzer model TOG-5050 measures total carbon (TC), inorganic carbon (IC), and total organic carbon (TOC). Its operation is based on the combustion / non-dispersive inferred gas analysis method widely employed for (TOC) measurements. Samples for (DOC) analysis were filtered (0.1 μm) and acidified (200 μl conc. nitric acid) and stored in 30 ml serum vials, capped with a butyl septa and crimp-sealed. Prior to analysis of samples, a standard was made

using potassium hydrogen phthalate. The standard curve consisted of solutions (10.0 μg/l, 20.0 μg/l, 30.0 μg/l and 40 μg/l). The method gives a linear response within sample range.

Particulate organic matter (POM) was collected from filtering known volumes of water sample through pre-weighed and combusted, 2.5 cm, glass fiber filters. Filters were weighed following numbering and ashing at 450° C for 1.5 hour. Ashing burned off any possible organic contaminants, the ashed filters were always handled with sterile stainless steel tweezers. Upon removal of the filters from the furnace, the filters were placed in a desiccator to cool. Subsequently, each filter was weighed and placed in a plastic scintillation vial, which had been weighed prior to use. The vials were then capped and numbered with filter numbers. Vials were pre-weighed to account for any filtrate material which could have been dislodged from the filter during travels back to the UK.

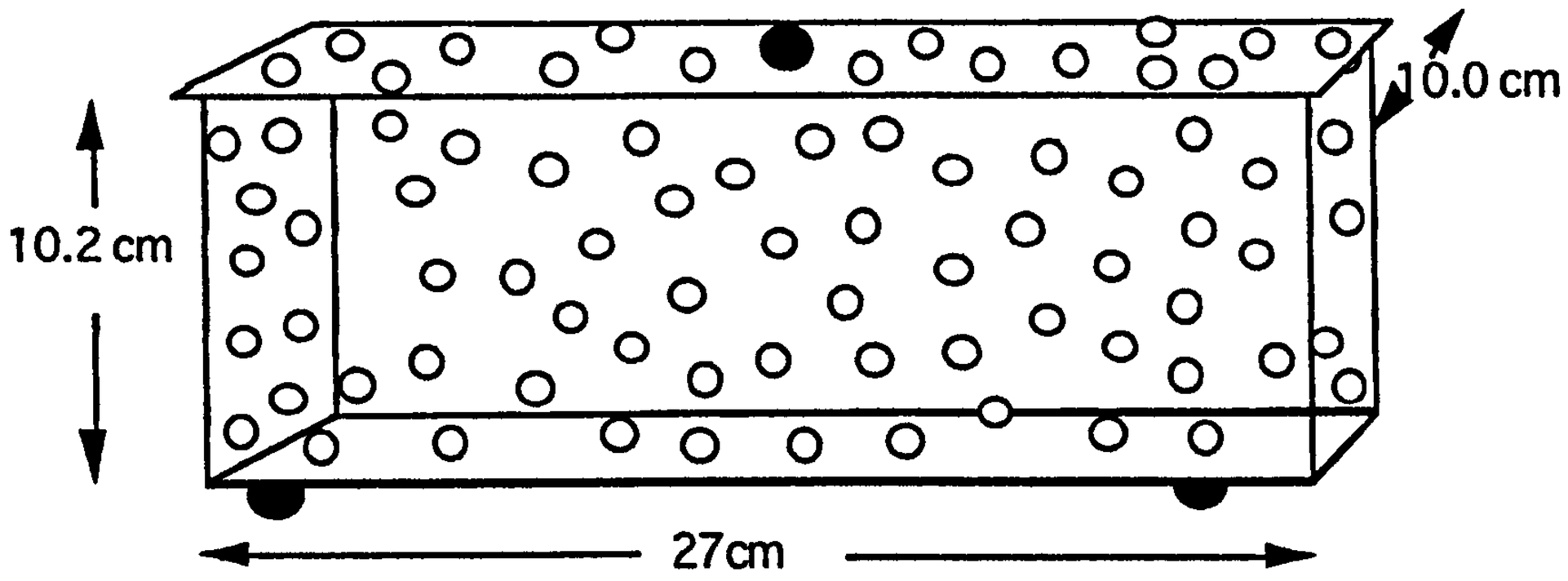


Figure 3.9 **Plexiglas drying chamber for (POM) filters**

After filtration, the filters were placed in a plexiglas box (Figure 3.9) which had an aluminum screen base and walls with numerous drying holes and a lid. Placed in the sun, the filters dried within 15 minutes and could be placed back into the numbered vials. Back at the University laboratory, vials, together with filters, were weighed; the filter was then weighed on its own (total particulate matter) and than ashed at 450° C for 1.5 hour and weighed again after cooling in a desiccator. The second weighing represented the inorganic fraction of the total (POM).

3:3:2 Alkalinity

Alkalinity was measured within 1 hour of sample collection. 25 ml of water sample was pipetted into a clean 100 ml flask and a few drops of BDH 4.5 indicator added. A solution of 0.01 M HCl was used to titrate the sample until there was a colour change

from blue to pale straw yellow, giving total alkalinity in ml. The concentration of the hydrochloric acid was standardized against 10 ml sodium carbonate titrated to pH 4.5.

Alkaline hardness as bicarbonate in mg L^{-1} =(ml 0.01 M Hcl used) x 24.4

Alkaline hardness as mg L^{-1} CaCO3 = (mL 0.01 M HCl used) x 20

3:4 Geological Analysis

3:4:1 XRD and SEM

Only one sample was analyzed on a Phillips (Norelco)-Model 12206/7 XRD at the University of Bristol. The sample was collected from Lucayan Caverns in February of 1996. Preparation of the sample was as follows:

1) A 2 cm section of the mud core was placed in a ceramic pestle and put in an oven at 37°C for 3 days to dry. Grinding was not necessary because of the nature of the mud being so fine.
2) This sample was than analyzed from 6.26, 3.29, 2.47 and 6.26 per minute. This setting would check for preserved iron in the sample.

Peaks were plotted on graph paper and interpolation of peak height was matched with known data to identify minerals.

SEM (Cambridge S250 Mk3 Stereroscan) photos were taken of the dried powder as well as of a section of a rock core collected from a wall within the Lucayan cavern sample site. Both samples were dried for 3 days in an oven at 37°C. Samples were then mounted onto a pedestal and placed in a gold sputter chamber and coated 3 times because of the extreme relief of the samples. They were than placed in the SEM chamber for viewing.

3:4:2 CHN&S

Samples were then analysed for CHN&S by the School of Chemistry, University of Bristol, Analytical Services. A mud core from Lucayan Cavern was sectioned (2 cm thick) dried in an oven (37° C) for 120 hours and ground with mortar and pestle.

3:4:3 Petrology

Rock samples collected via coring and hand sampling, were cut into thin (15x30x10 mm) chips for microscopic analysis, for cutting with a water lubricated saw. To preserve the original vertical orientation, a small notch was cut into the edge of the sample. The chips were then placed on an aluminum covered Fisher Corning hot plate to dry at 225°C. Drying time took approximately 20 to 30 minutes. Following the mixing of Petropoxy 154 with the proper amounts of blue dye and curing agent, the epoxy was poured over the hot chips till they were completely covered. Curing time was approximately 10 minutes.

After the epoxy was dry and the chips were cool enough to handle, each sample was then ground on a water cooled diamond grinder. All sides of a chip were ground until the surface was smooth enough to stick to a wet clean slide. At this point a mountable surface had been produced. The chips were allowed to dry overnight. The following morning they were glued to glass slides with an ultraviolet-sensitive glue (curing time was about 5 minutes). Prior to glueing, the glass slides were frosted on the mounting side with a grinder and then wiped down with ethanol. Finished slides were then processed on a Buelhler 38-1450-160 Petro-Thin section machine.

4

Field Trip One (February 1994) Results for Lucayan Caverns and Owl's Hole, Grand Bahama

Introduction

Samples were collected on two separate occasions at the same sample site in the Lucayan Caverns (appendix 1), once in the Owl's Hole (appendix 2) and once in the Stargate Blue Hole. The results of the two Lucayan field trips are presented as they provide information about possible changes with time, February 1994 and February 1996. In addition, between trips, collection methods were improved. One of the main differences was that samples were obtained in the Lucayan Caverns in February of 1994 over several days, whereas samples collected in February of 1996 were collected all during one dive. Determinations of salinity, temperature, pH, alkalinity, dissolved oxygen, dissolved organic carbon, and volatile fatty acids were made on all water samples along with direct bacterial populations and potential activity measurements. Direct bacterial population assessments were only done on the first field trip.

In addition, in 1998 a Data Sonde 3, water quality multiprobe sensor from Hydrolab Austin, Texas, was used in all sample sites for this project. The ability of this probe to measure *in situ* and frequently; in this case, every two seconds, provided invaluable data for comparison and confirmation of data collection methods used in the 94/96 field trips.

4:1
(Lucayan Caverns 1994)

4:1 Geochemistry

Geochemical analysis of water column samples enabled effective characterization of each site and also provided a guide for bacterial and other sampling. *In situ* salinity measurements made it possible to determine the boundaries of the fresh water lens. Location of the major density interfaces allowed effective placement of the sample tubes used in water collection. Analysis of these samples made it possible to recognize the

potential bacterial role in altering the water column chemistry and the bacterial populations within cave walls.

4:1:1 Salinity

The water column can be divided into three major bodies of water on the basis of salinity (Figure 4.1). The first body, the fresh water lens which in most part is located within the ceiling rock, begins at an unknown depth (possibly sea level) and ends just above 14 m. The biological definition for fresh water is water having a salinity between 0 g/l to 0.5 g/l (Standard Methods for the Examination of Water and Wastewater, 16th edn., 1985). For description purposes for this study, the fresh water body will be defined as the unit of water found above the upper mixing zone boundary (UMB) at 14 m. Salinity within this first body of water increased from 0.93 g/l, from the first measured depth 13 m, to 9.51 g/l at the UMB at 14 m; an increase in salinity of 8.58 g/l over a vertical distance of just under 1 m.

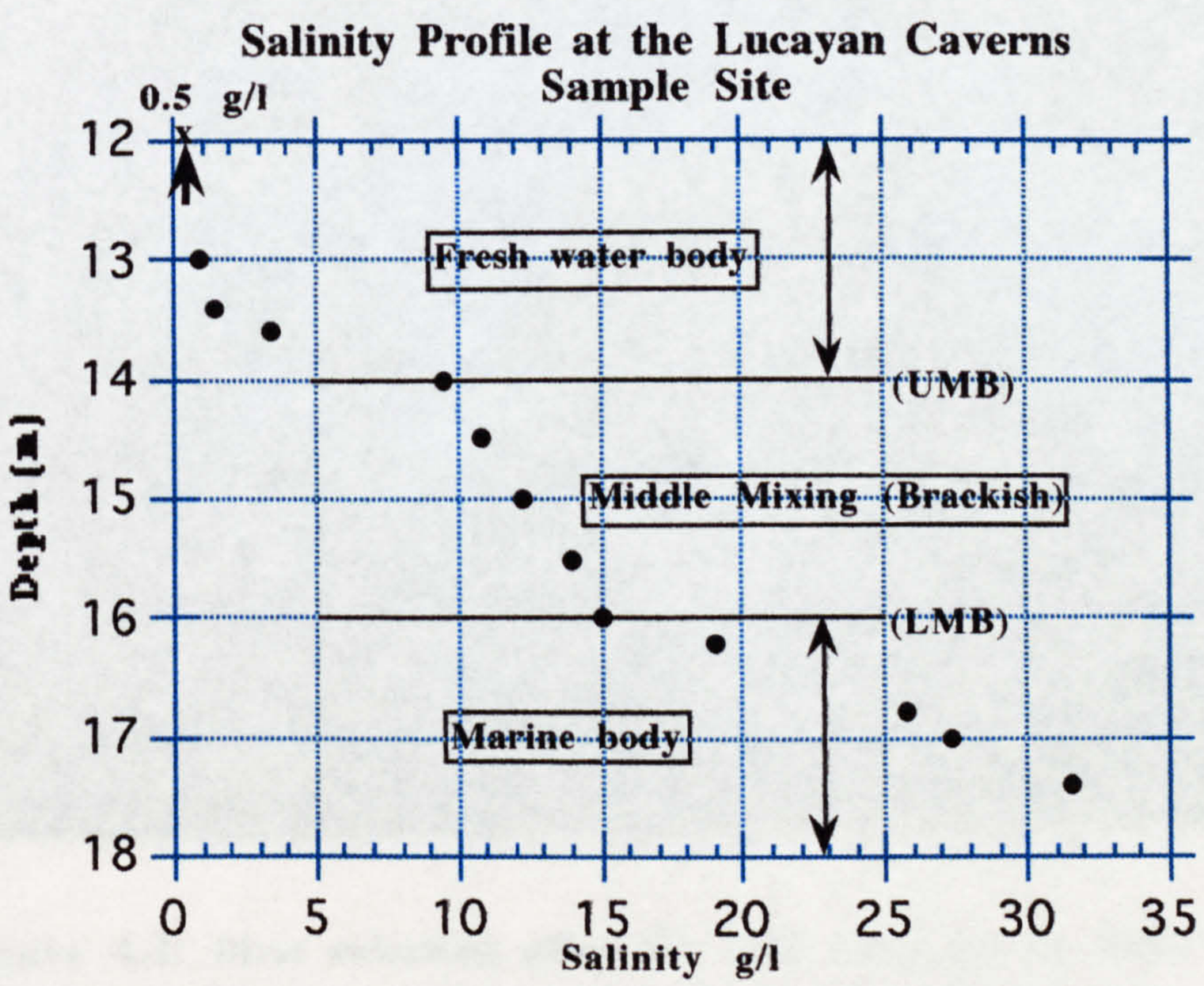


Figure 4.1: (1994) Salinity profile through the water column in Wedding Hall, Lucayan Caverns.

The UMB was defined as the salinity boundary at 14 m. The middle mixing zone (MMZ) was the area between the 14.1 m to 15.9 m; where salinity gradually increased

from 9.5 g/l to 14 g/l at 15.5m; (the salinity at 15.9 m was not defined). The lower mixing zone LMB was the boundary at 16 m. The marine body was defined as the region beginning just beneath the LMB and continues to depth. The average salinity of the fresh water body is an estimated 1.94 g/l. The average salinity for the MMZ is 11.69 g/l. The UMB has a salinity of 9.51 g/l and is a significant transition zone between fresh water body and the slightly brackish water below (Figure 4.2(Photo)). Hovering above the UMB the interface appears cloudy. Moving through the UMB into the MMZ, the water continues to mix, creating a shimmer throughout the MMZ. Once through the LMB clear vision is restored.

A general description of passage morphology in the Lucayan Caverns is given in (Figure 4.3) to demonstrate the difficulty in finding sites where divers have excess, at one site, to the full salinity range (i.e., fresh to salt water). Figure 4.3 also gives the general location of sites relative to the entrance.

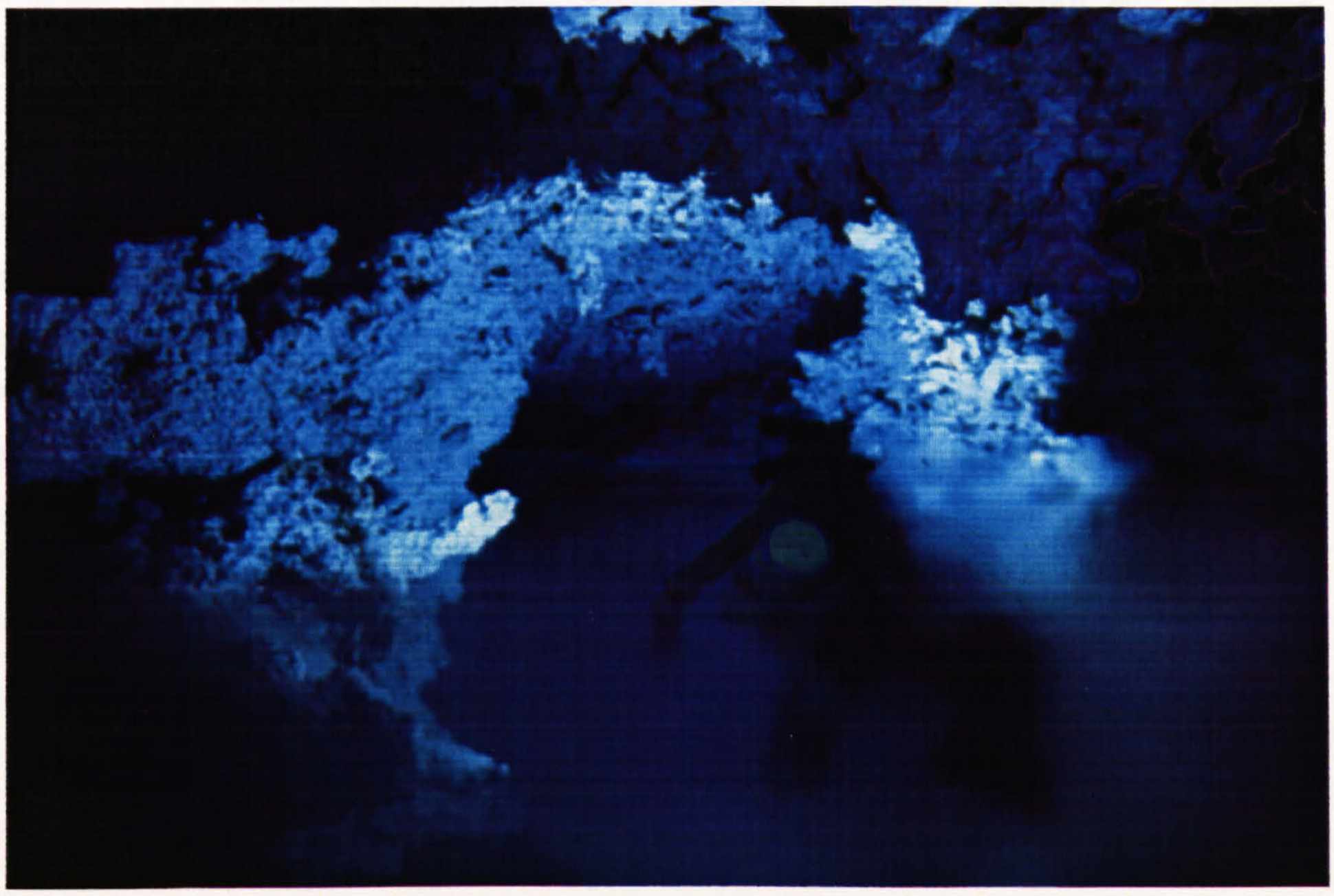


Figure 4.2: Diver swimming above the UMB which can be clearly seen as a white cloudy layer beneath the diver. Notice the lower portion of the diver is out of focus. Notice also the rough nature of the cave wall within the mixing zone; the zone at and beneath the clouds.

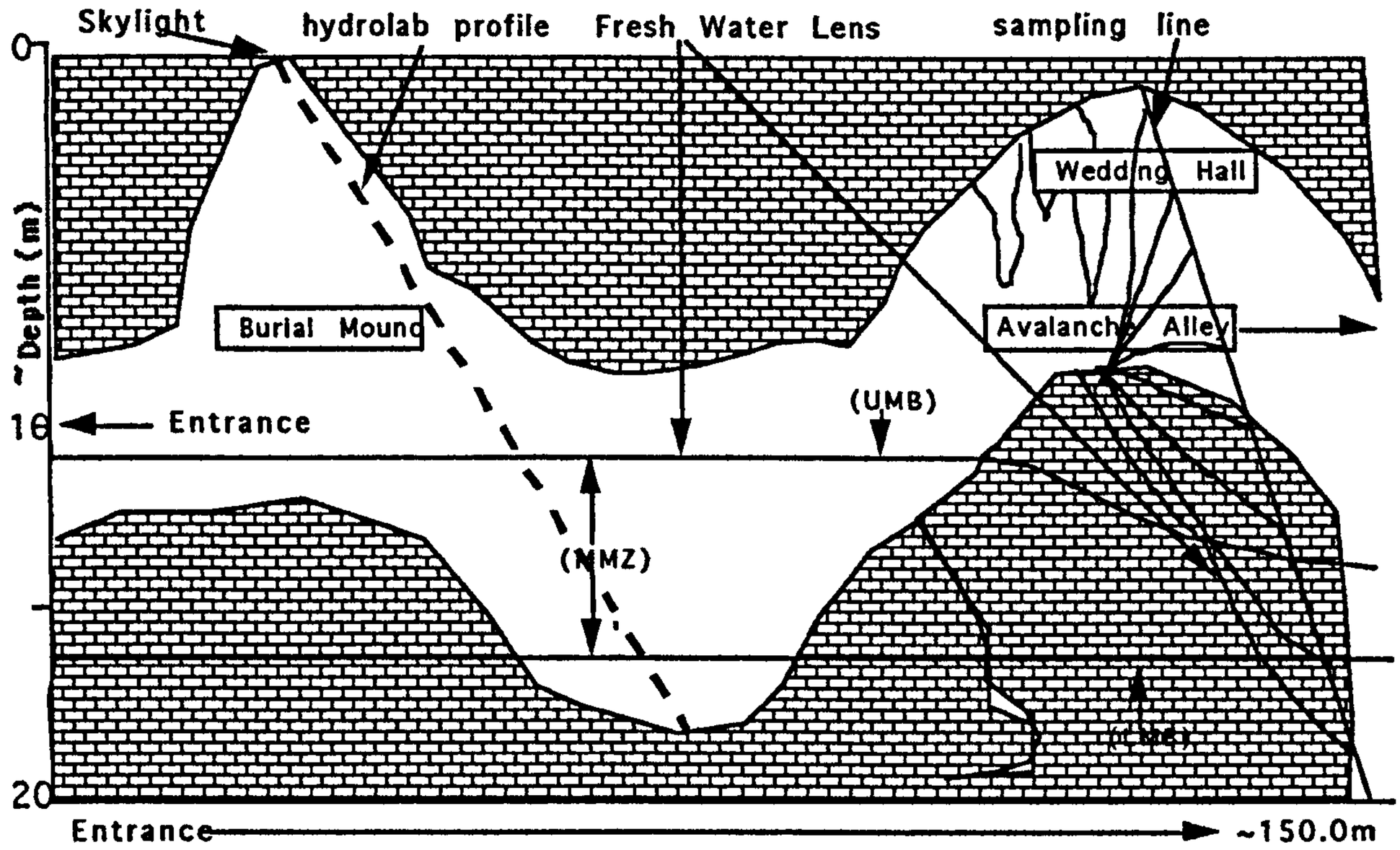


Figure 4.3. Section of Lucayan Caverns known as Burial Mound and Wedding Hall Room (not to scale).

A reason for making the distinction between the two sites seen in Figure 4.3, is that the water column profile differs between these two sections despite being part of the same cave system, only about 150 m apart and hydrologically open to each other. Salinity profiles (Figure 4.4; 4.5) and other geochemical profiles taken within Burial Mound and Wedding Hall using the hydrolab illustrate the need for caution when trying to generalize the geochemical distributions within caves, i.e., the UMB is not at 14 m throughout the cave, and in the Burial Mound site is at appoximately 11.5 m. However, the hydrolab results clearly demonstrate the reproducibility of the 1994 salinity profiles at the Wedding Hall site (Figure 4.1 & 4.5).

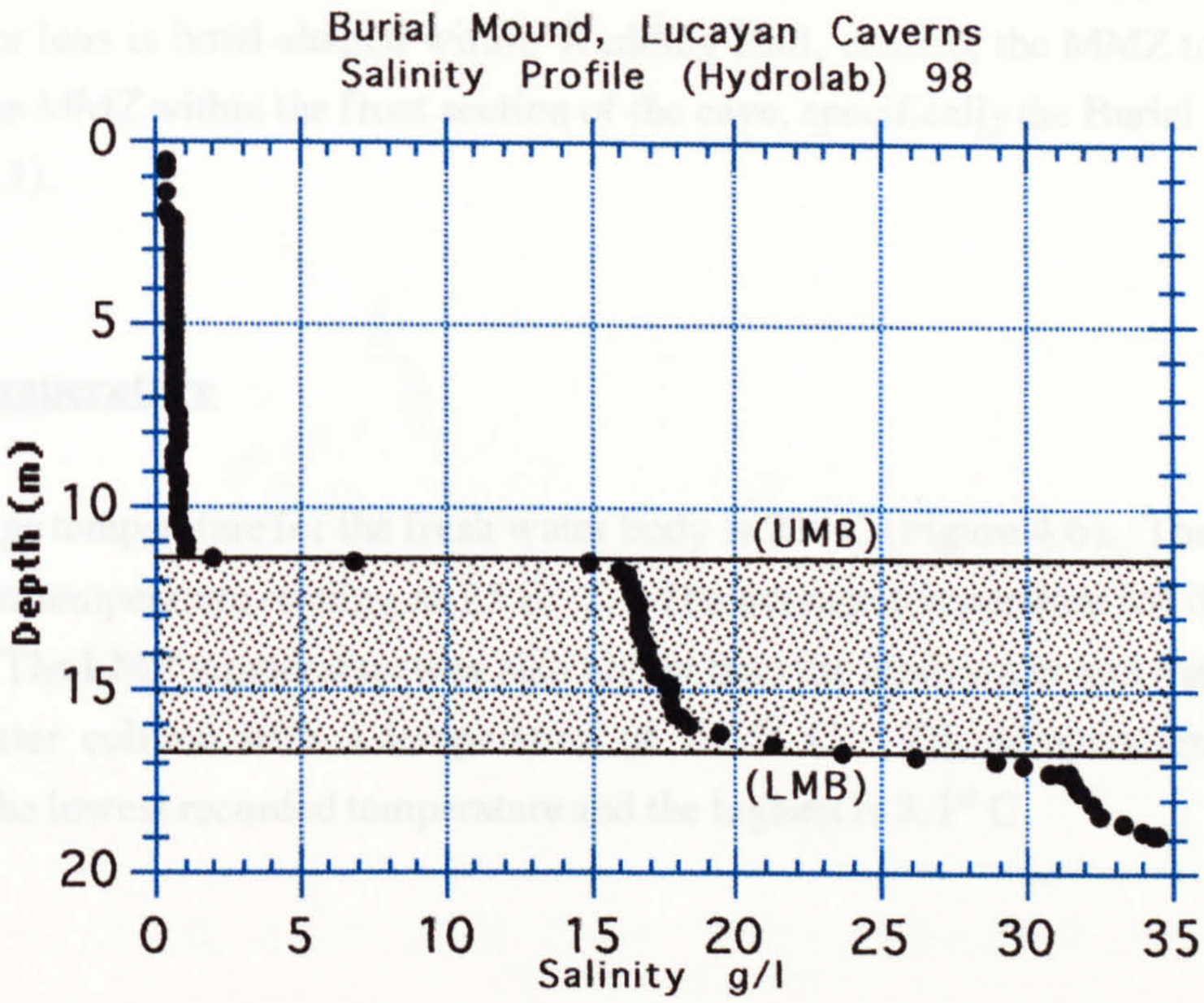


Figure 4.4 **Burial Mound Salinity Profile (Hydrolab). Compare figure 4.4 with 4.5, a salinity profile from Wedding Hall. The mixing zone within Wedding Hall is almost half as thick as that found in the Burial mound room.**

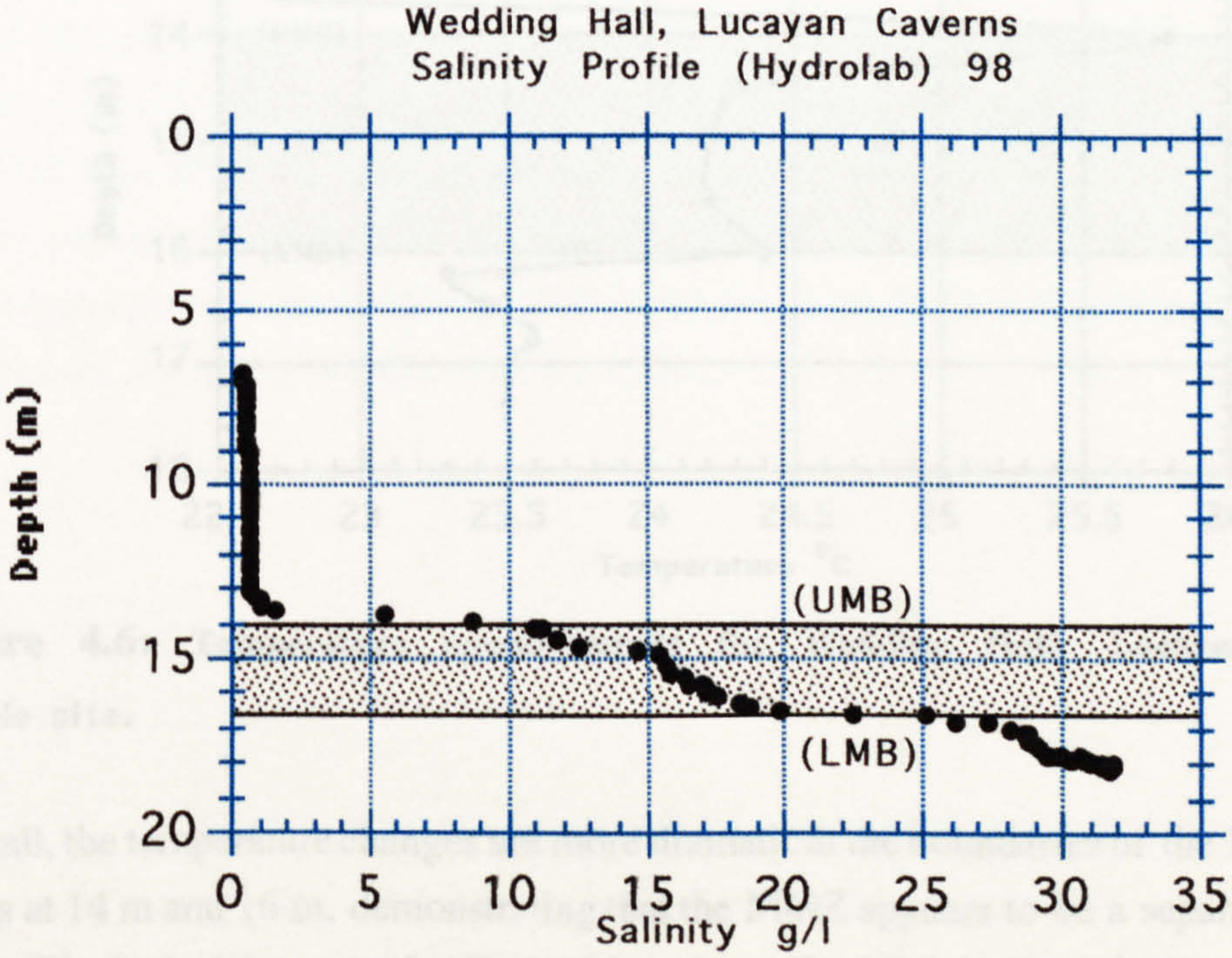


Figure 4.5 **Salinity profile using the hydrolab for Wedding Hall, Lucayan Caverns.**

However, the LMB in 1998 is at approximately 16.5 m in both sites suggesting that the fresh water lens is bowl-shaped within Wedding Hall, causing the MMZ to be half as thick as the MMZ within the front section of the cave, specifically the Burial Mound site (Figure 4.3).

4:1:2 Temperature

The average temperature for the fresh water body is 23° C (Figure 4.6). The UMB had the highest temperature reading at 25.8° C. The average temperature for the MMZ is 24.6° C. The LMB temperature was also higher than the fresh water and marine section of the water column with a temperature of 24.4° C. The temperature difference between the lowest recorded temperature and the highest is 3.1° C.

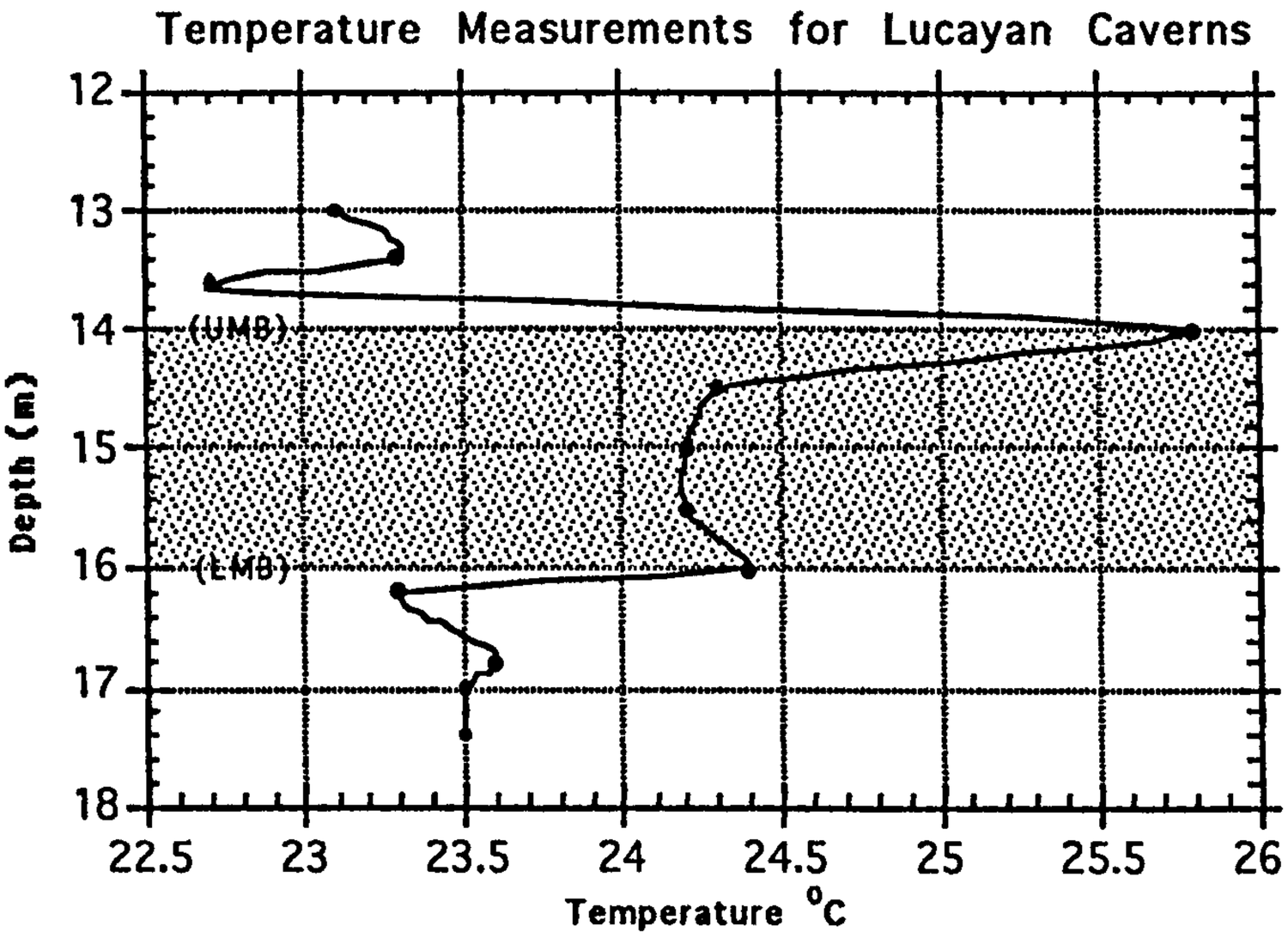


Figure 4.6: Temperature measurements for Wedding Hall, Lucayan Caverns sample site.

Overall, the temperature changes are more dramatic at the boundaries of the MMZ, with peaks at 14 m and 16 m, demonstrating that the MMZ appears to be a separate body of water. The hydrolab results for Burial Mound and Wedding Hall are similar to the hand sample results for 1994 in that temperature increases at the UMB and well into the MMZ.

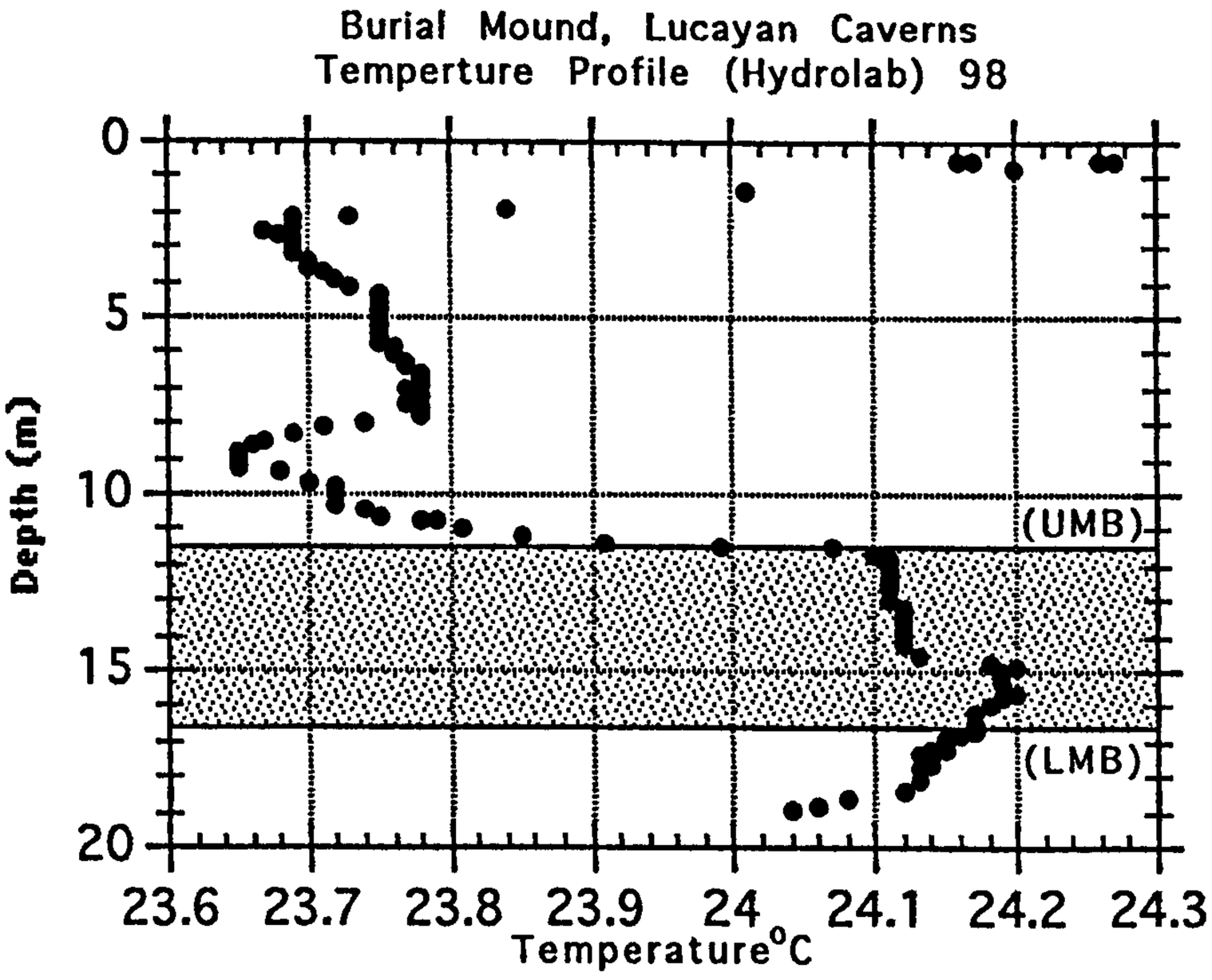


Figure 4.7: Burial Mound hydrolab temperature profile

Looking more closely at the temperature differences between Burial Mound and Wedding Hall hydrolab profiles, (Figure 4.7; 4.8) the temperature at 6.5 m within Burial Mound is 23.7°C where in Wedding Hall the temperature at 6.5 m is slightly warmer, 24.1°C. Shallower profiles could be collected with the hydrolab because the hydrolab was small enough to reach into places where divers could not go. The same applies to deeper samples. At 16.5 m, the depth of the (LMB) within Burial Mound, the temperature was recorded at 24.1°C and at the same depth within Wedding Hall the temperature was not much different at 24.2°C.

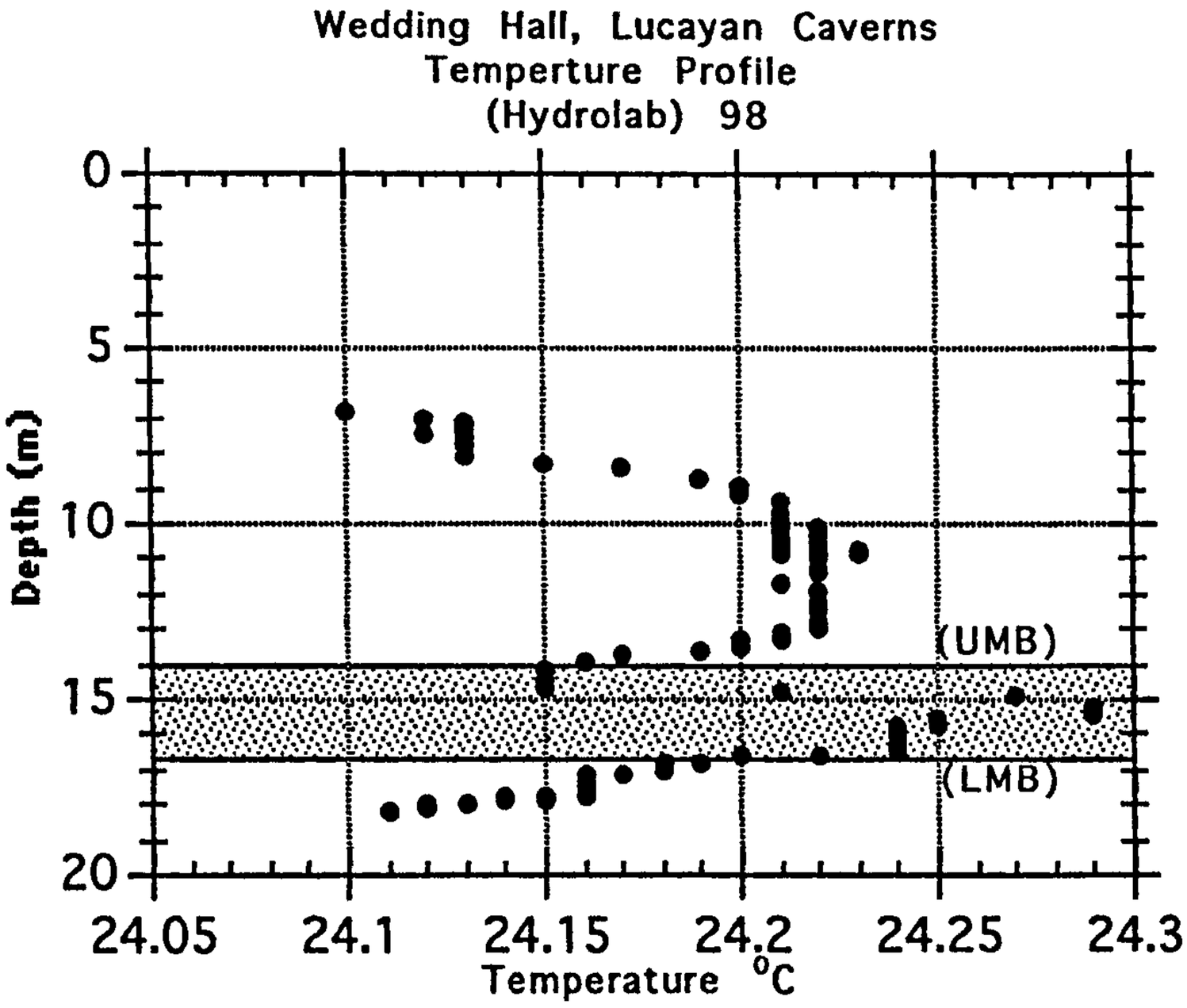


Figure 4.8: Wedding Hall hydrolab temperature profile

Although the initial comparison between the hydrolab temperature profile (Figure 4.8) and the manual temperature profile (Figure 4.6) is different, the trends are similar when ± 1 m tidal fluctuation is taken into account and in the case of the 1994 sample, a slight warming up of the sample once out of the cave could have occurred. Although the lab was set up in the shade, the difference between the *in situ* temperature and open air would have been about 1-3°C warmer. Another factor which would could have influenced the temperature of the water sample was that the cell, used to measure temperature and to take other measurements, would also have been warmed by air temperature. Since the volume of the water cell was 286 ml ±1 ml, it is possible that some absorption of heat could have occurred in this instance.

4:1:3 pH Hydrogen-Ion Activity

The fresh water body is the most alkaline portion of the water column with an average pH of 7.35 (Figure 4.9). The pH changes are relatively small, (ca 0.15), through out the water column. Within the MMZ the pH values are relatively consistant thoughout at pH 7.2. In the most saline section of the water column at depth, the pH values are seemingly becoming more alkaline. Maximum change in pH value appears to happen at the mixing zone boundaries, a change which is also seen in the hydrolab profile (Figure 4.11). Pin-point changes, such as what is observed at the UMB do not instill much

trust in the data point, but two hydrolabs of the same identical model and both calibrated prior to field runs produce identical results. However, compared to the pH changes that occur at the UMB in some Mexican caves of 2 to 3 pH units (Tom Iliffe personal communication), these pH changes are still large.

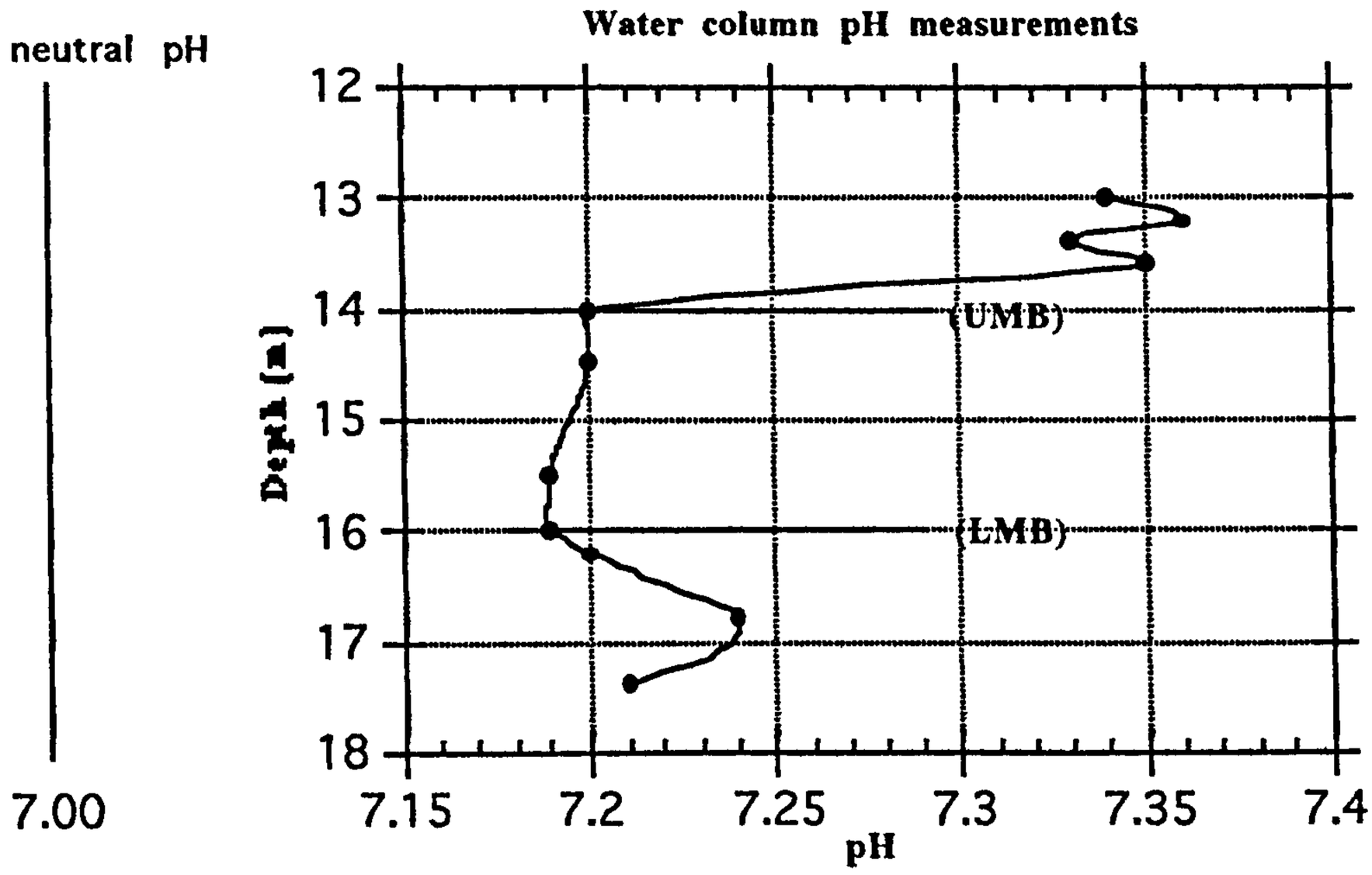


Figure 4.9: Lucayan Caverns pH measurements.

In light of the data presented here, together with *in-situ* (i.e., the brittle cave wall and the intensity of the UMB interface) the observed pH changes are no surprise.

The hydrolab pH measurements for both Burial Mound and Wedding Hall are different. Looking at Burial Mound first, (Figure 4.10) at 6.5 m the reading was pH 8.2 and at the same depth in Wedding Hall, pH 7.12 (Figure 4.11). At 16.5 m, the LMB for both sites, Burial Mound had a reading of pH 7.84 whereas in Wedding Hall at the same depth, the measurement was more acidic (pH 6.9).

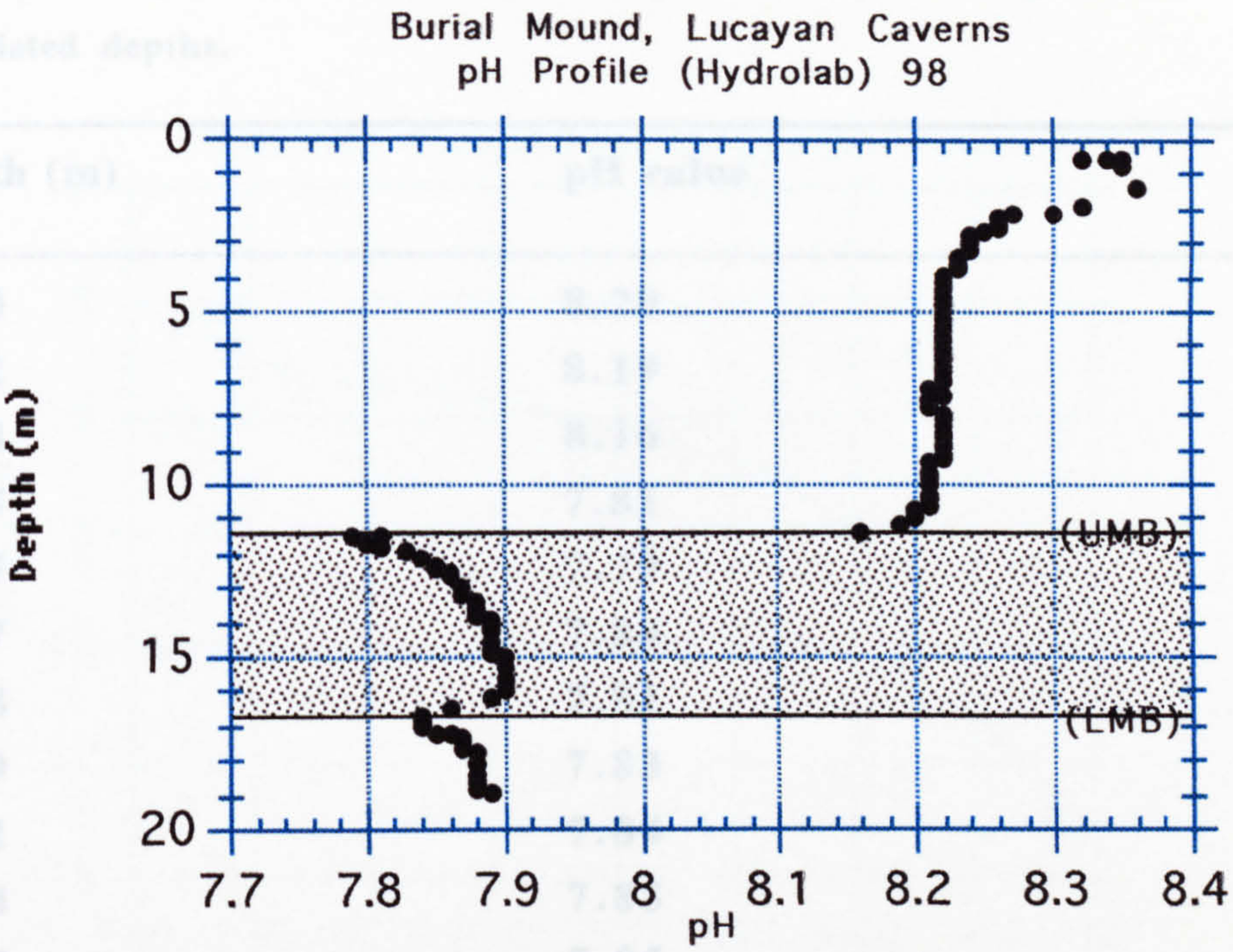


Figure 4.10: Burial Mound pH hydrolab profile

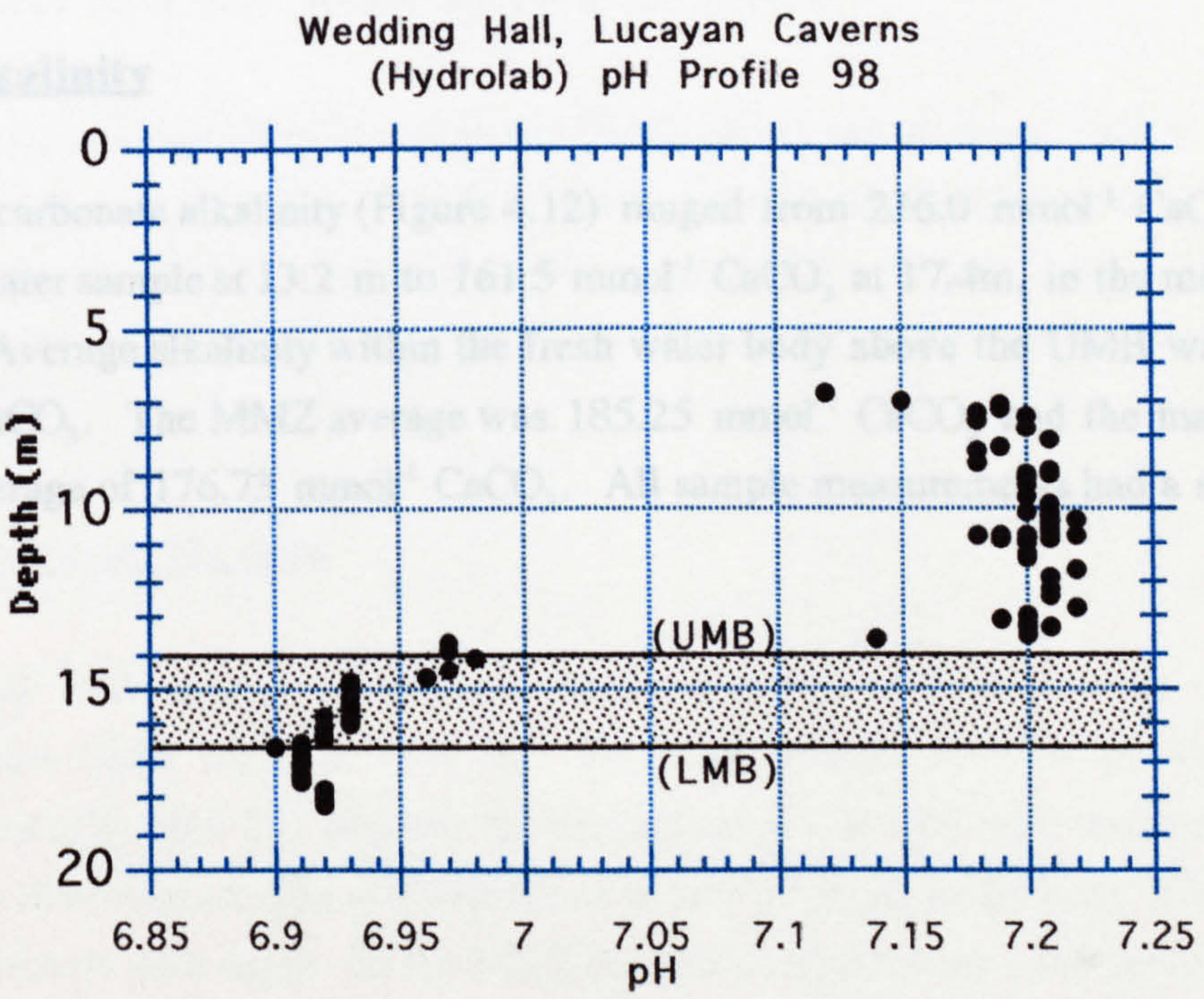


Figure 4.11: Wedding Hall pH hydrolab profile

Table 4.1: Hydrolab values for pH changes over depth for figure 4.10 along with associated depths.

Depth (m)	pH value
11.0	8.20
11.2	8.19
11.4	8.16
11.5	7.81
11.5	7.79
11.7	7.80
11.8	7.81
11.9	7.83
12.1	7.84
12.3	7.85
12.5	7.85

4:1:4 Alkalinity

Bicarbonate alkalinity (Figure 4.12) ranged from 236.0 $mmol^{-1}$ $CaCO_3$ in the freshest water sample at 13.2 m to 161.5 $mmol^{-1}$ $CaCO_3$ at 17.4m, in the most marine sample. Average alkalinity within the fresh water body above the UMB was 231.30 $mmol^{-1}$ $CaCO_3$. The MMZ average was 185.25 $mmol^{-1}$ $CaCO_3$ and the marine body had an average of 176.73 $mmol^{-1}$ $CaCO_3$. All sample measurements had a std ± 0.03 n=3.

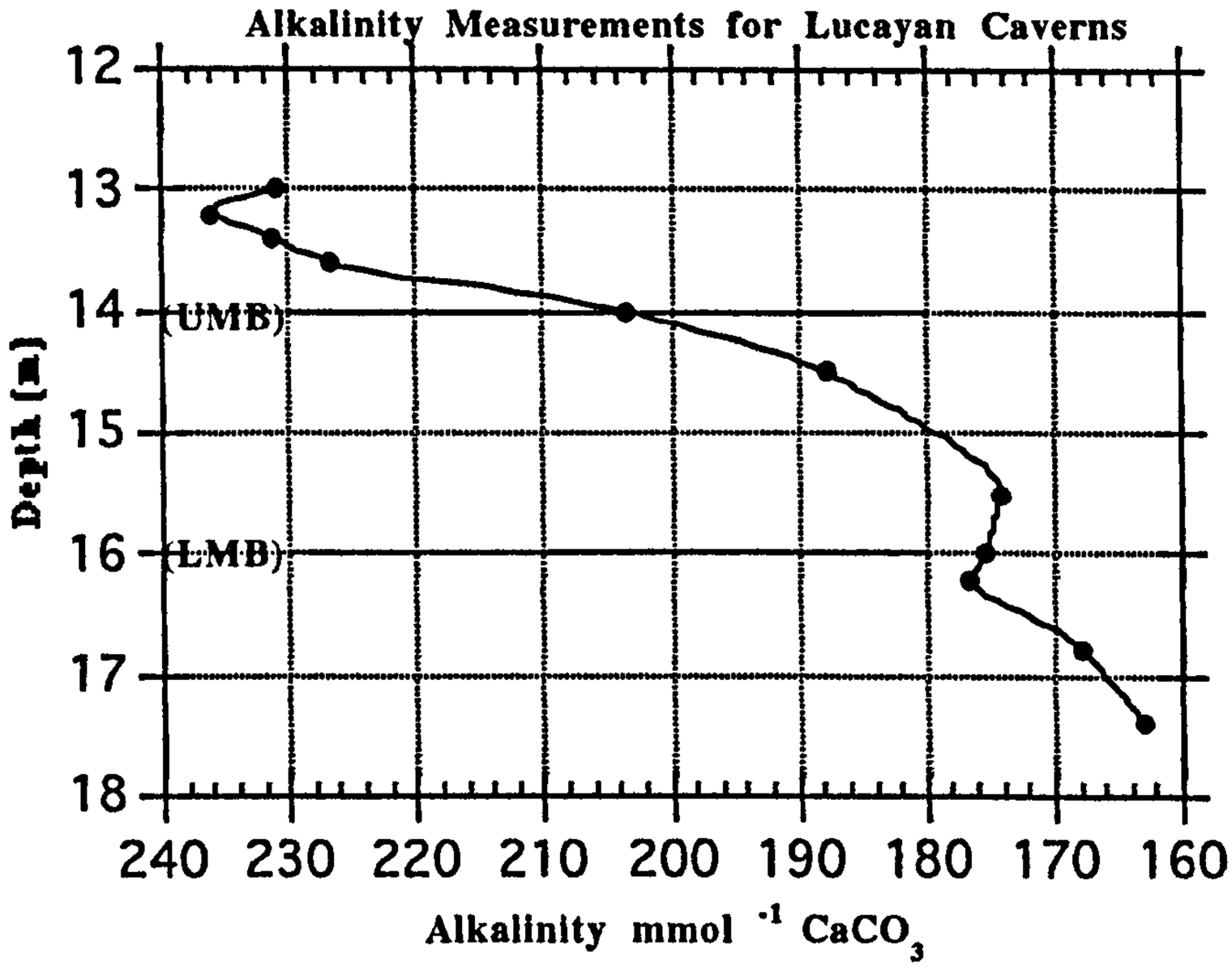


Figure 4.12: Alkalinity results for Lucayan Caverns.

The most rapid changes occur through the UMB (14 m) and the upper part of the MMZ (15.5m). From 15.5 m to the LMB, alkalinity was relatively stable, but in the saline zone, below the LMB, alkalinity increased gradually. Again, the MMZ values demonstrate its uniqueness as a separate body of water within the water column.

4:1:5 Dissolved Oxygen

The highest concentration of oxygen (4.92 mg/l) was at 13.4 m with the lowest concentration (0.99 mg/l) at 16.8 m. The water sample at 13.4 m was already undersaturated by 40% DO whereas the deepest sample at 16.8 was undersaturated by 90%. The dissolved oxygen concentrations within the fresh water body (Figure 4.13) decrease linearly with depth. In the MMZ oxygen concentrations were relatively stable and decreased only gradually by 2 mg/l over a two metre distance.

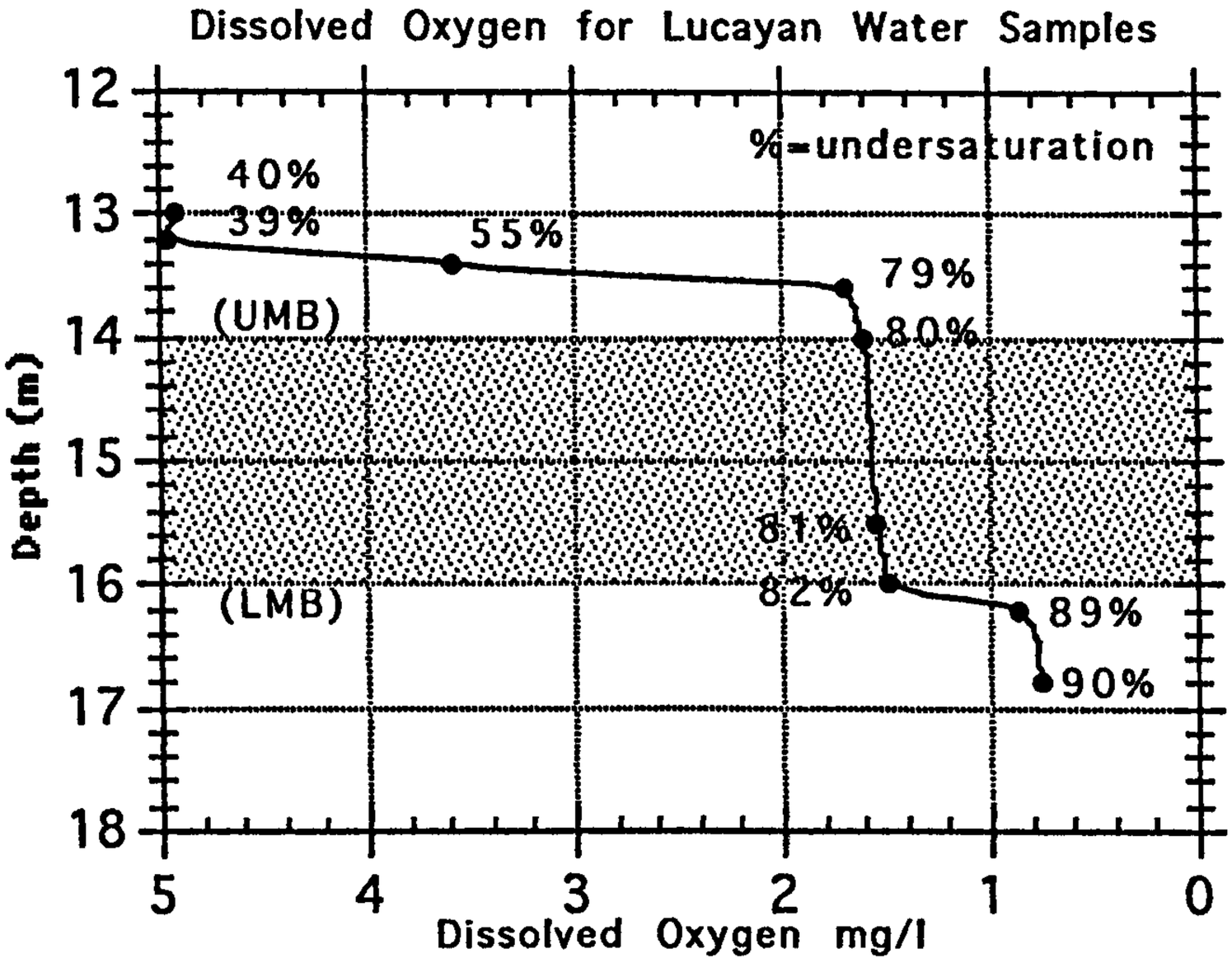


Figure 4.13: Wedding Hall Site percent dissolved oxygen.

Once through the LMB, the oxygen concentrations decreased over a short distance but then stabilised below 16.2 m. The average oxygen concentration within the fresh water body was 4.49 mg/l, in the MMZ the concentration was 1.58 mg/l. The saline zone had an average oxygen concentration of 0.87 mg/l. The most stable oxygen concentrations were within the MMZ, and again, the geochemistry results demonstrate the presence of a different body of water from measurements taken at the UMB and the LMB. What is noticably different between Figure 4.13 and the hydrolab measurements of the same site (Figure 4.15) is the difference between the DO values from the (UMB) and the (LMB). The drop in DO percent is not as significant within the manual profile but significant within the hydrolab profile. Whether these changes are based on seasonal or weather patterns or maybe even tital cycles is not known at this time.

The hydrolab profiles for Burial Mound and Wedding Hall (Figure 4.14 and 4.15) differ from each other where at 6.5 m in Burial Mound there is noticeably less dissolved oxygen (4.5 mg/l) compared to a reading of 6.2 mg/l DO at 6.5 m in Wedding Hall.

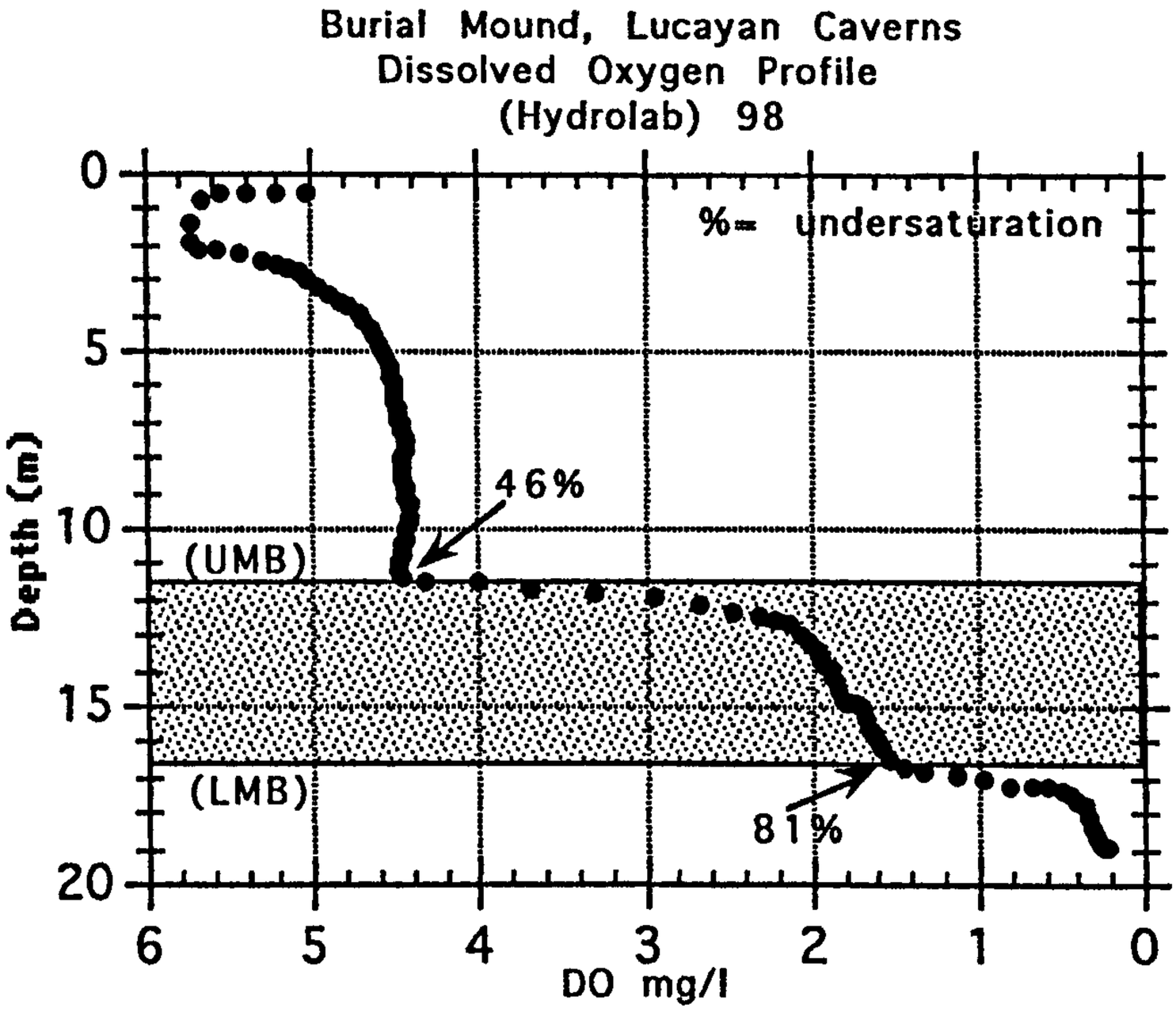


Figure 4.14: Burial Mound hydrolab oxygen profile

Comparing the Wedding Hall hydrolab results with the 1994 results is difficult. There are so few data points in the 1994 set and it has been recognized through use of the hydrolab that dramatic changes can occur over very short distances. The 1994 DO trend is similar to the hydrolab results demonstrating decreased DO with depth, however,

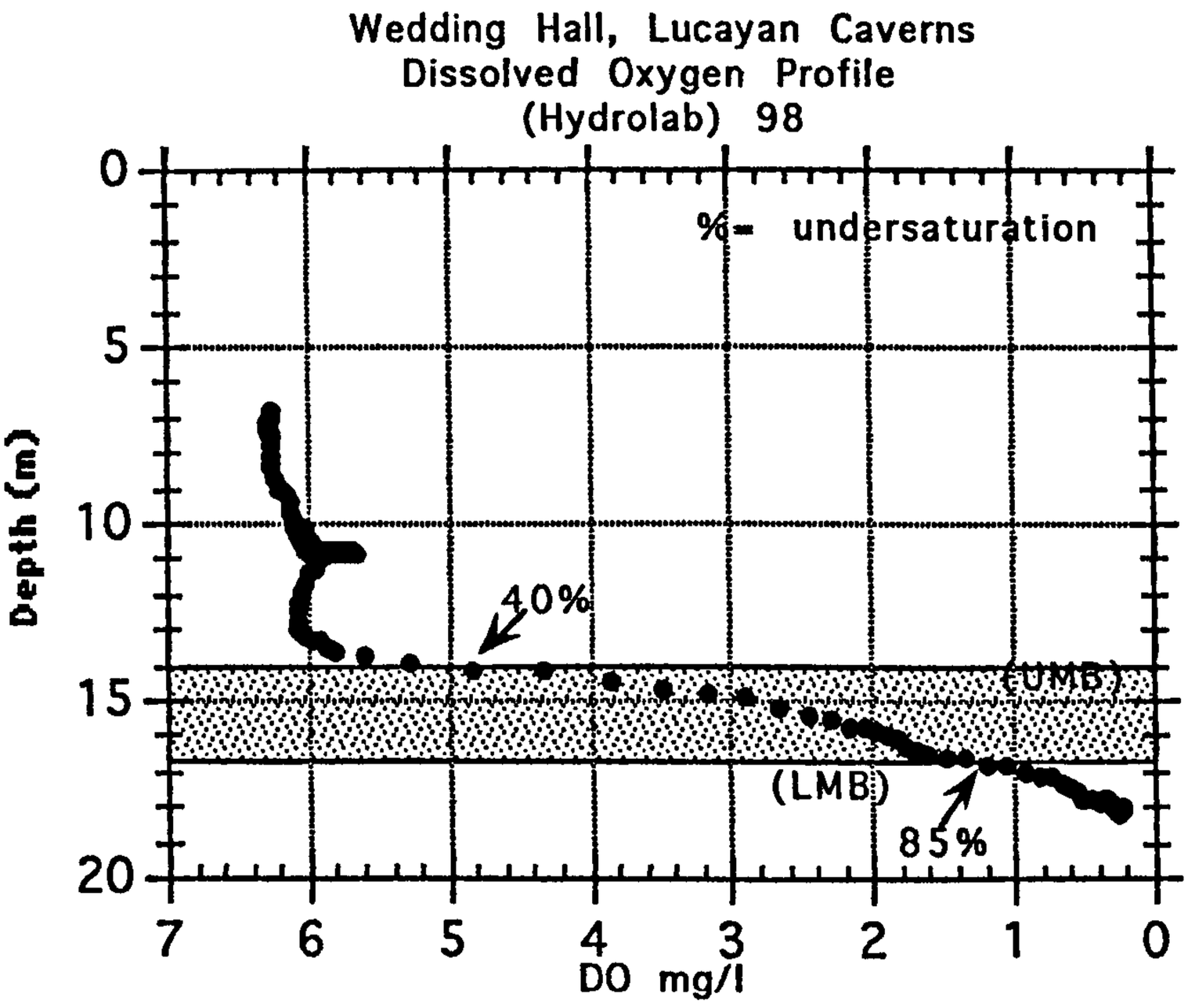


Figure 4.15: Wedding Hall hydrolab oxygen profile

using the 14 m depth sample for comparison, the 1994 levels within that water sample was 1.6 mg/l whereas in the hydrolab profile, at the exact same depth, the DO value ranged between 4.4 mg/l to 5.4 mg/l. At 16.5 m in Wedding Hall, the DO result for 1994 was about 0.8 mg/l. The hydrolab result for Wedding Hall at 16.5 m was 1.4mg/l and at the same depth for Burial Mound the hydrolab result was, 1.8 mg/l. Overall, the body of water within Wedding Hall is more oxygenated than Burial Mound.

Comparing all the hydrolab profiles taken within the Lucayan Caverns, differences (Figure 4.16; A,B,C,D) can be seen in water chemistry from the entrance of Lucayan (Burial Mound Room) to the furthest penetration, (Avalanche Alley). Examining the salinity (Figure 4.16 A)

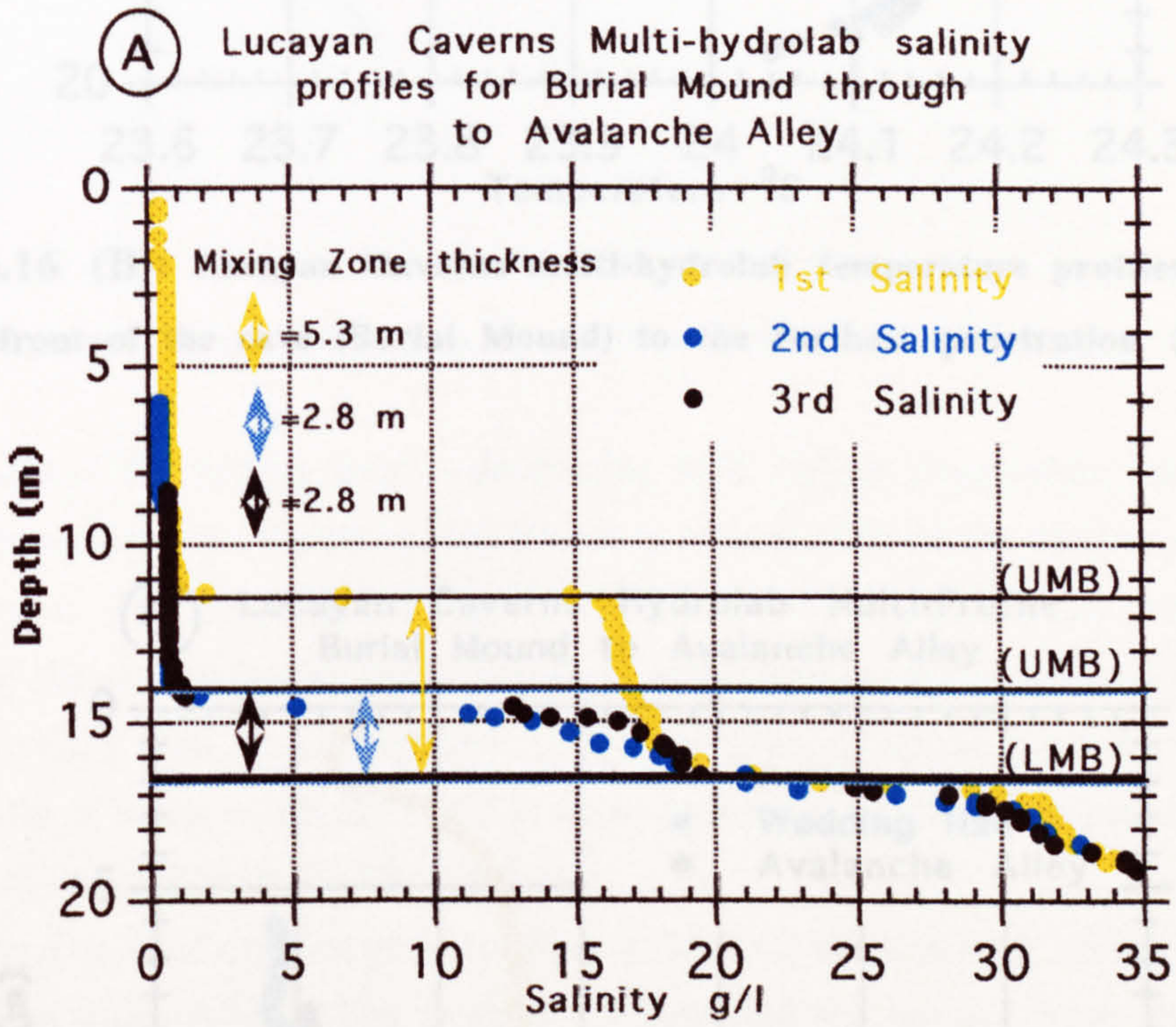


Figure 4.16 (A): Lucayan Caverns multi-hydrolab salinity profiles, starting from the front of the cave (1st Burial Mound), (2nd Wedding Hall) to the furthest penetration (3rd Avalanche Alley)

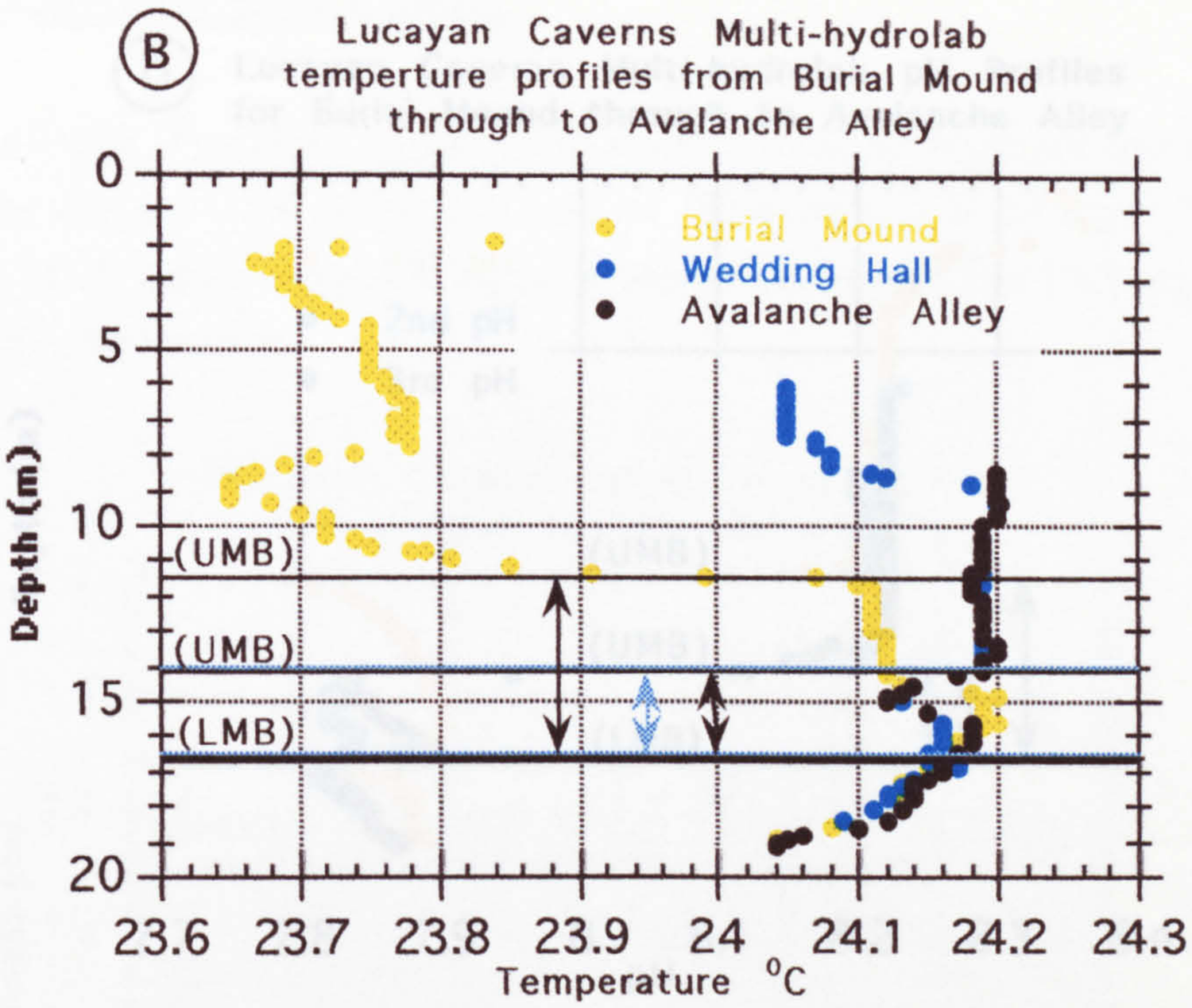


Figure 4.16 (B): Lucayan Caverns multi-hydrolab temperature profiles, starting from the front of the cave (Burial Mound) to the furthest penetration (Avalanche Alley)

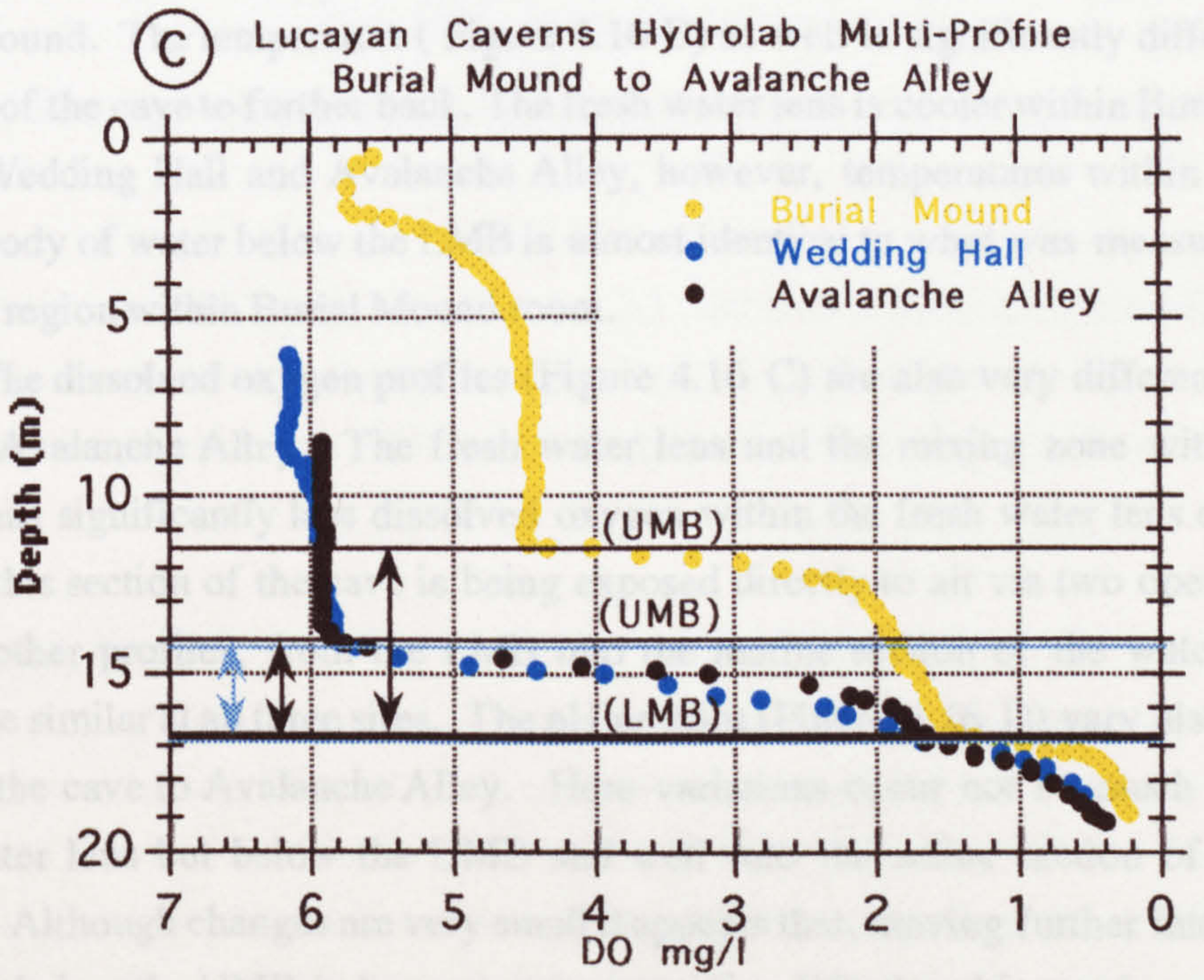


Figure 4.16 (C): Lucayan Caverns multi-hydrolab DO profiles, starting from the front of the cave (Burial Mound) to the furthest penetration (Avalanche Alley)

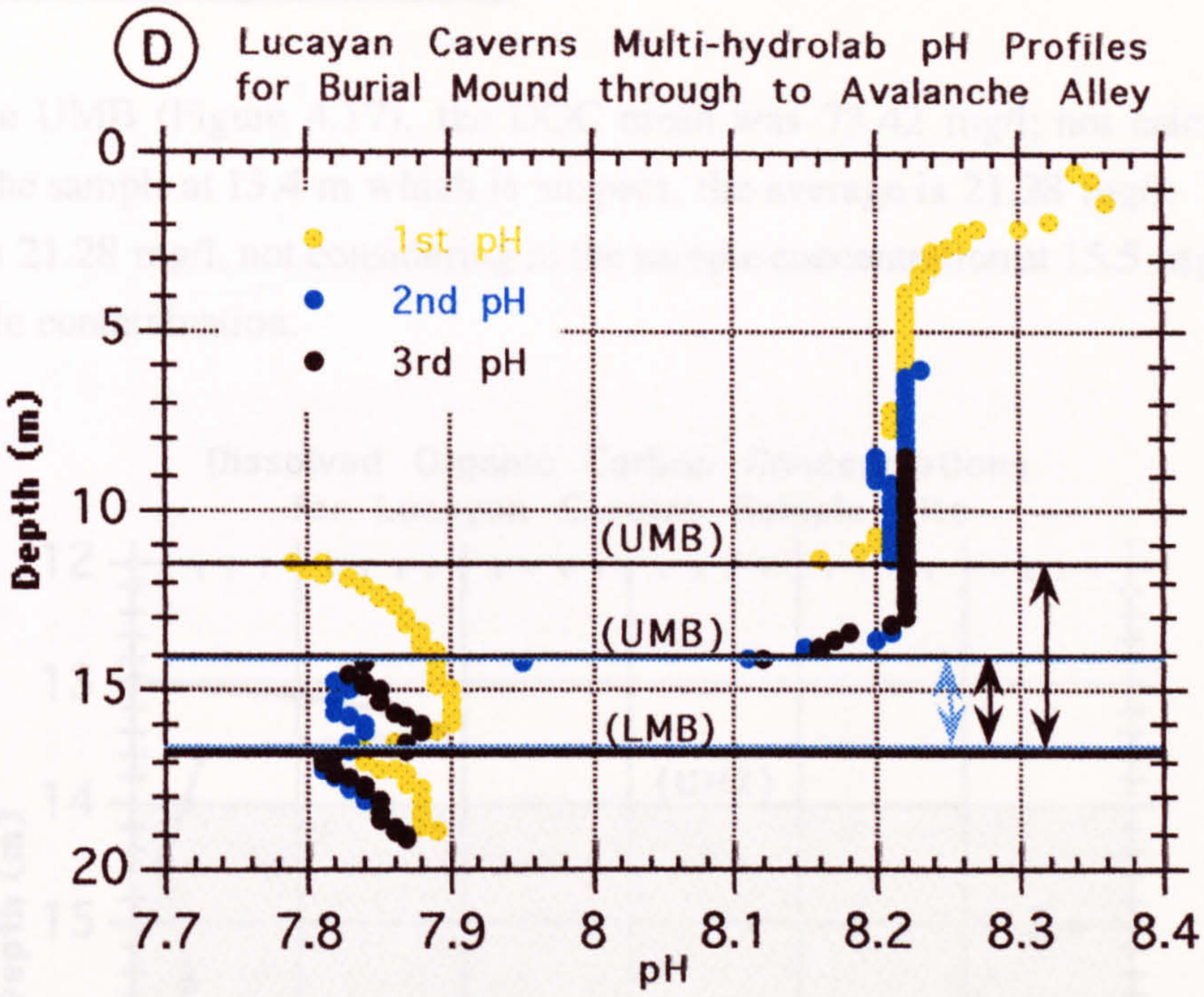


Figure 4.16 (D): Lucayan Caverns Multi-hydrolab pH profiles, starting from the front of the cave (Burial Mound) to the furthest penetration (Avalanche Alley)

the thickness of the mixing zone is decreasing with further penetration into the cave. The mixing zone within Wedding Hall Room is almost 50% of the mixing zone within Burial Mound. The temperature (Figure 4.16 B) as well is significantly different from the front of the cave to further back. The fresh water lens is cooler within Burial Mound than in Wedding Hall and Avalanche Alley, however, temperatures within the MMZ and the body of water below the LMB is almost identical to what was measured within the same region within Burial Mound room.

The dissolved oxygen profiles (Figure 4.16 C) are also very different from the front to Avalanche Alley. The fresh water lens and the mixing zone within Burial Mound has significantly less dissolved oxygen within the fresh water lens despite the fact that this section of the cave is being exposed directly to air via two openings. As seen in other profiles, from the LMB into the marine section of the water column, values are similar at all three sites. The pH profiles (Figure 4.16 D) vary also from the front of the cave to Avalanche Alley. Here variations occur not so much within the fresh water lens but below the UMB and well into the saline section of the water column. Although changes are very small it appears that, moving further into the cave, the water below the UMB is becoming more acidic. Whether this trend continues into the cave is something that will be investigated at a later date.

4:1:6 Dissolved Organic Carbon

Above the UMB (Figure 4.17), the DOC mean was 73.42 mg/l; not calculating the value of the sample at 13.4 m which is suspect, the average is 21.28 mg/l. The MMZ mean was 21.28 mg/l, not considering in the sample concentration at 15.5 mg/l because of possible contamination.

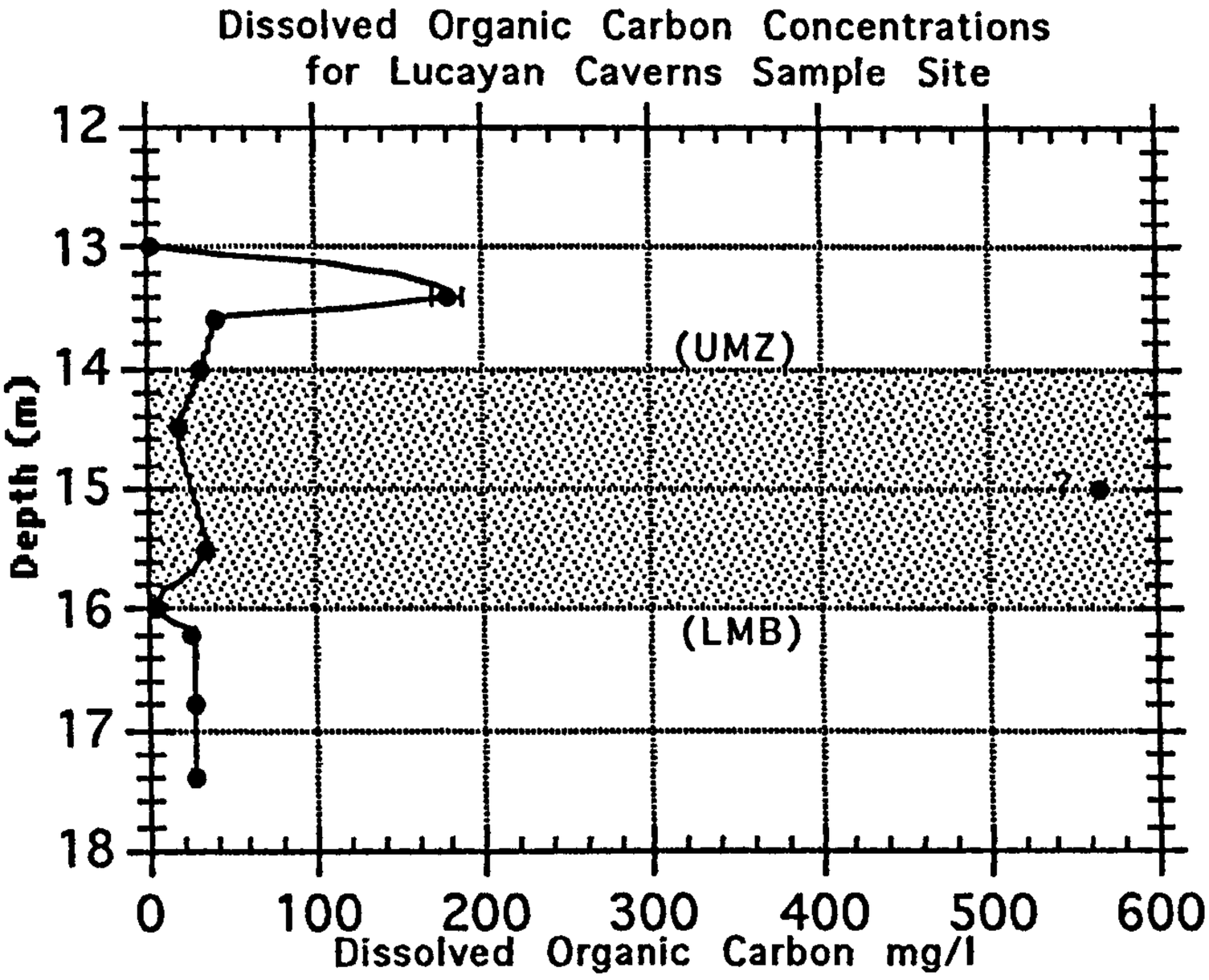


Figure 4.17: Dissolved organic carbon measurements for Lucayan Caverns 1994 field trip.

The marine body DOC mean was 21.94 mg/l, not much more than the MMZ mean. DOC concentrations, surprisingly, appear not to reflect the presence of density interfaces at 14 m and 16 m. DOC is relatively consistent throughout the water column.

4:1:7 Total Acetate

Acetate concentrations (Figure 4.18) overall were relatively low, ranging from 0.4 μM/ml at 13.4 m to a maximum concentration of 33.8 μM/ml at 16.8 m. Distributions were quite variable with increases below the UMB and through the LMB. Average concentrations above the UMB were 5.3 μM/ml, in the MMZ were 13.3 μM/ml, and below the LMB were 23.2 μM/ml, showing that the acetate concentrations were increasing with depth.

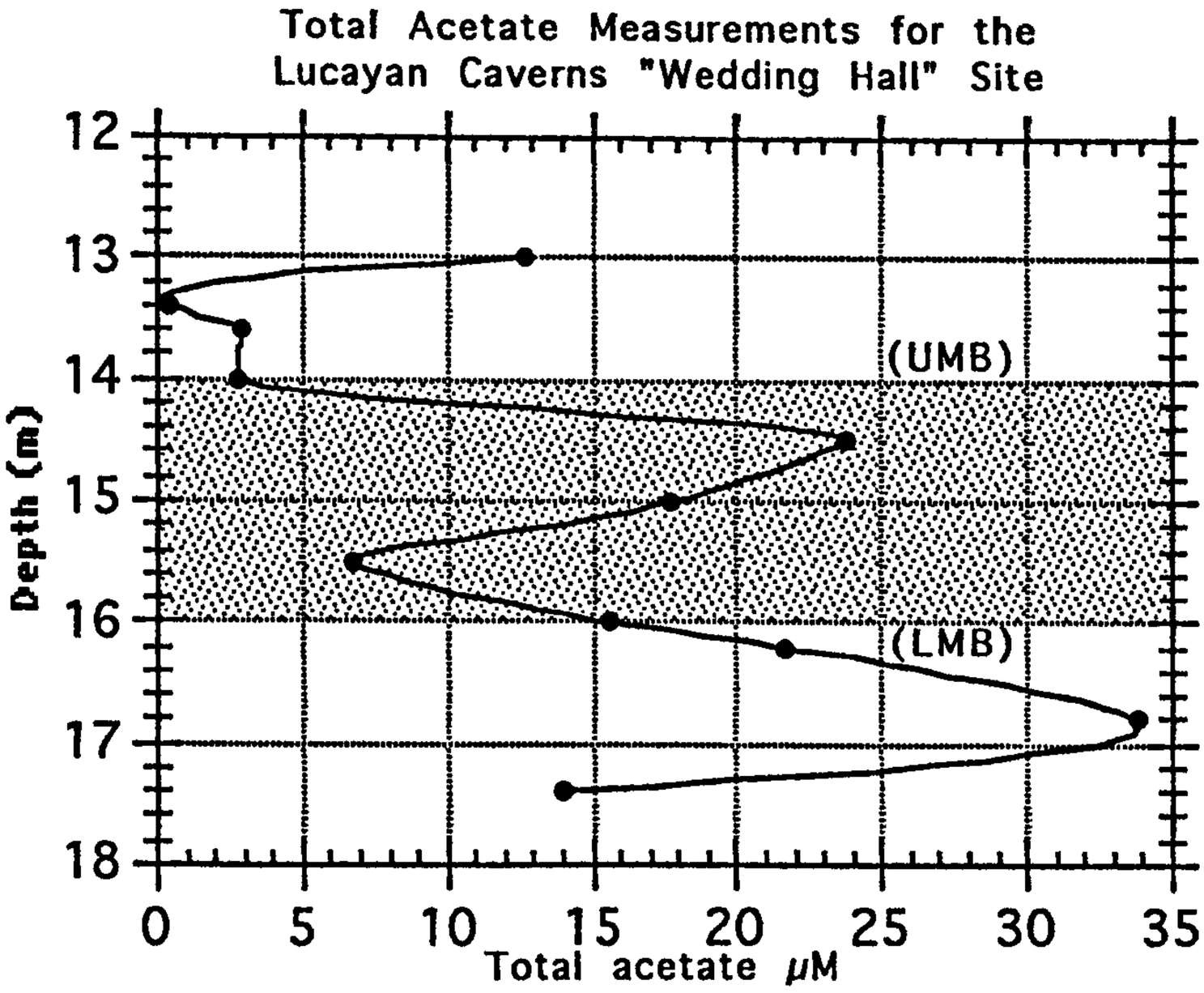


Figure 4.18: Total acetate concentrations within the water column in Lucayan Caverns Wedding Hall sample site.

4:2 Radiotracer Results

4:2:1 ^{14}C Acetate

Turnover rates for radiolabeled ^{14}C acetate were positive for activity in all water samples (Figure 4.19). Activity measurements ranged for the two-hour incubations, between 0.1 μM/hr. at 13 m to a maximum of 4.1 μM/hr. at 16.8 m. For the six-hour-incubation, minimum activity and maximum activity was at the same depth as was the two-hour incubation with 0.1 μM/hr. at 13 m and 4.8 μM/hr. at 16.8 m.

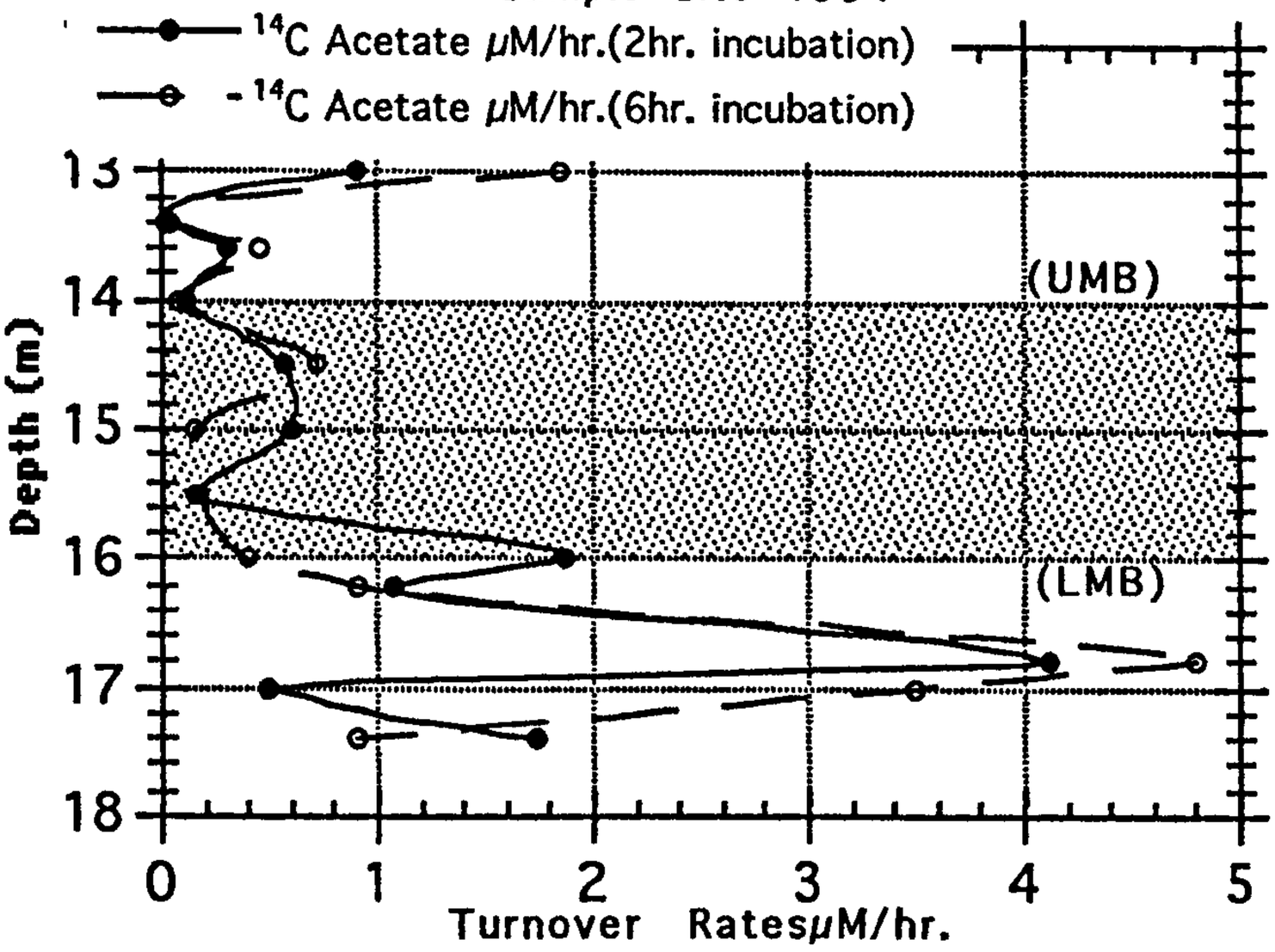


Figure 4.19: Estimated turnover rates for radiolabeled ^{14}C acetate in the Lucayan Cavern sample site.

Average turnover rates for the two-hour incubations in the fresh water body was 0.41 μM/hr.; the MMZ, 0.66 μM/hr., and in the marine body, 1.86 μM/hr.. For the six-hour incubations the average fresh water body concentration was 0.77 μM/hr.; the MMZ, 0.30 μM/hr., and in the marine body, 2.52 μM/hr. Based on both, the two-hour and the six-hour incubation results, it can be seen that the turnover rates are increasing with depth, and maximum activity occurs at 16.8 m for both.

4:2:2 ^{14}C Bicarbonate Utilization

The ^{14} C bicarbonate incorporation results (Figure 4.20), although not high, reflect the presence of autotrophic bacteria able to synthesize organic carbon from CO_2. Approximately 98% to 99% of the radiolabeled isotope was not utilized, demonstrating that incorporation was not tracer-limited. The two-day incubation results illustrate that maximum activity occurs at the UMB at 14.0 m with a value of 79.19 μmol^{-1}/day. The minimum activity was at 13.4 m in the fresh water body with a value of 23.01 μmol^{-1}/day. On average the two-day

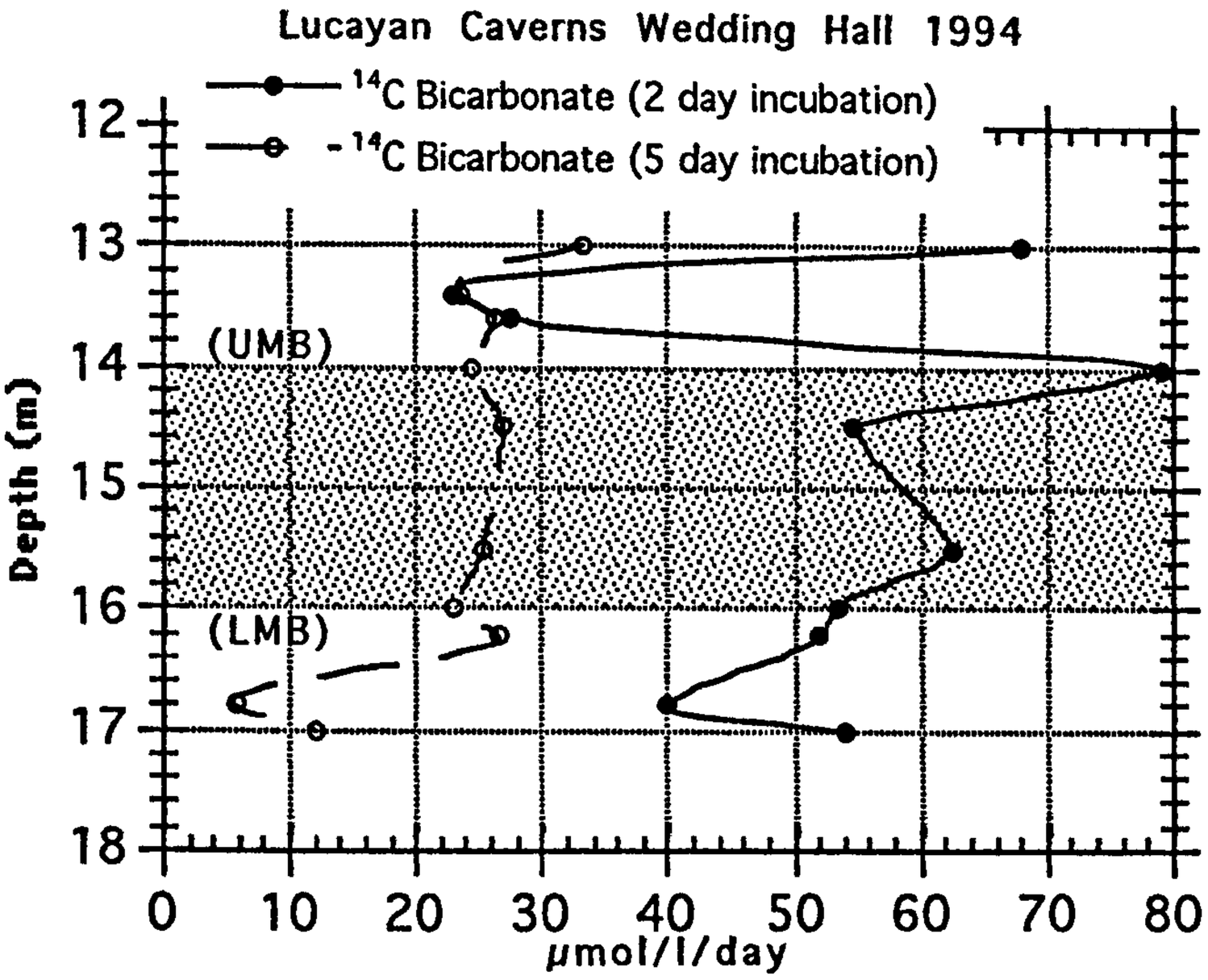


Figure 4.20: ^{14}C Bicarbonate estimated incorporation rates / day.

rates for the fresh water body was 39.5 μmol^{-1}/day, 62.41 μmol^{-1}/day within the MMZ, and 48.64 μmol^{-1}/day within the marine body, hence, the maximum mean activity occurred within the MMZ. The reason for this is unclear, but since acetate turnover results for the different incubation periods were very similar it might reflect changes specific to chemoautotrophic activity, such as depletion of reduced inorganic substrates in the samples now separated from other water masses.

The five-day incubation rates are almost 50% less than the two-day rate. The average rate for the fresh water body was 27.7 μmol^{-1}/day, 24.90 μmol^{-1}/day for the MMZ, and 14.68 μmol^{-1}/day for the marine body. The five-day incubation rates demonstrate that the most active region of the water column is the fresh water body, followed by the MMZ, and then the marine body. The most varied activity occurred within the fresh water body and the marine body. Once at the UMB and into the MMZ, measurements were very stable for the five-day incubations but not for the two-day rates. In contrast, the two-day results were varied throughout the vertical sampling site.

4:2:3 Sulphate Reduction

The reduction of $^{35}SO_4^{2-}$ into totally reduced inorganic sulphide (TRIS) was used to determine sulphate reduction rates within this sample site (Figure 4.21). Although the

amounts of sulphate reduction were very low, the results clearly demonstrated the presence of this process at this site despite the presence of oxygen.

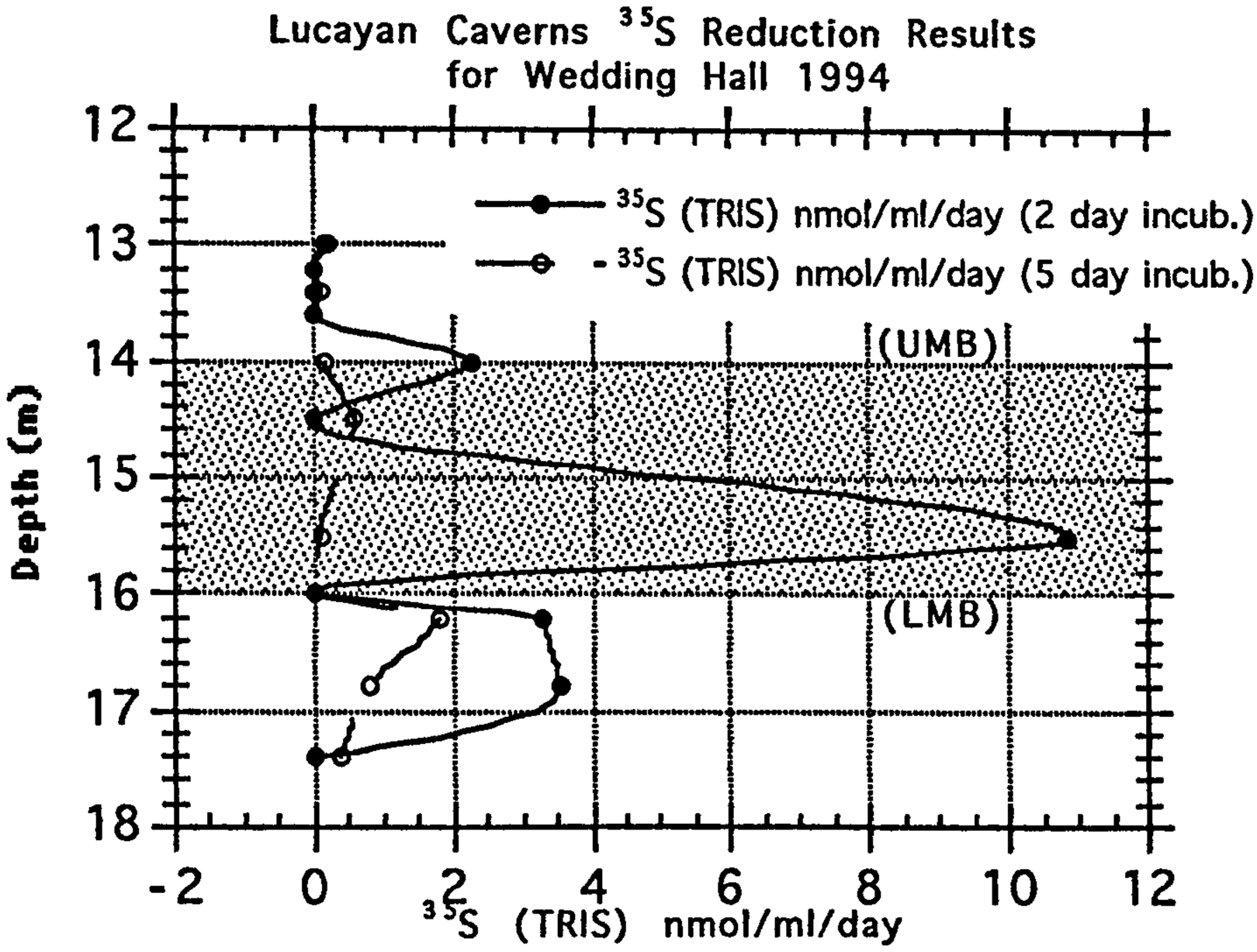


Figure 4.21: Potential ^{35} S sulphate reduction rates for the Lucayan Caverns sample site

Again, the two day incubation rates were substantially higher than the five-day rates. Both the 2-day and the 5-day measurements were either very low or negative within the fresh water body. Where O_2 concentrations were highest (Figure 4.13) within the MMZ activity was the greatest for both incubation periods, along with the samples from the marine body. The average turnover rate for the 2-day incubation for the fresh water body was 0.06 nmol/ml/day, the MMZ, 3.28 nmol/ml/day, and the marine body, 1.77 nmol/ml/day. The fresh water results for the five-day incubations were as follows; fresh water, 0.07 nmol/ml/day, 0.53nmol/ml/day for the MMZ, and for the marine body, 0.56 nmol/l/day.

The turnover data plotted against oxygen mg/l (Figure 4.22) illustrate that

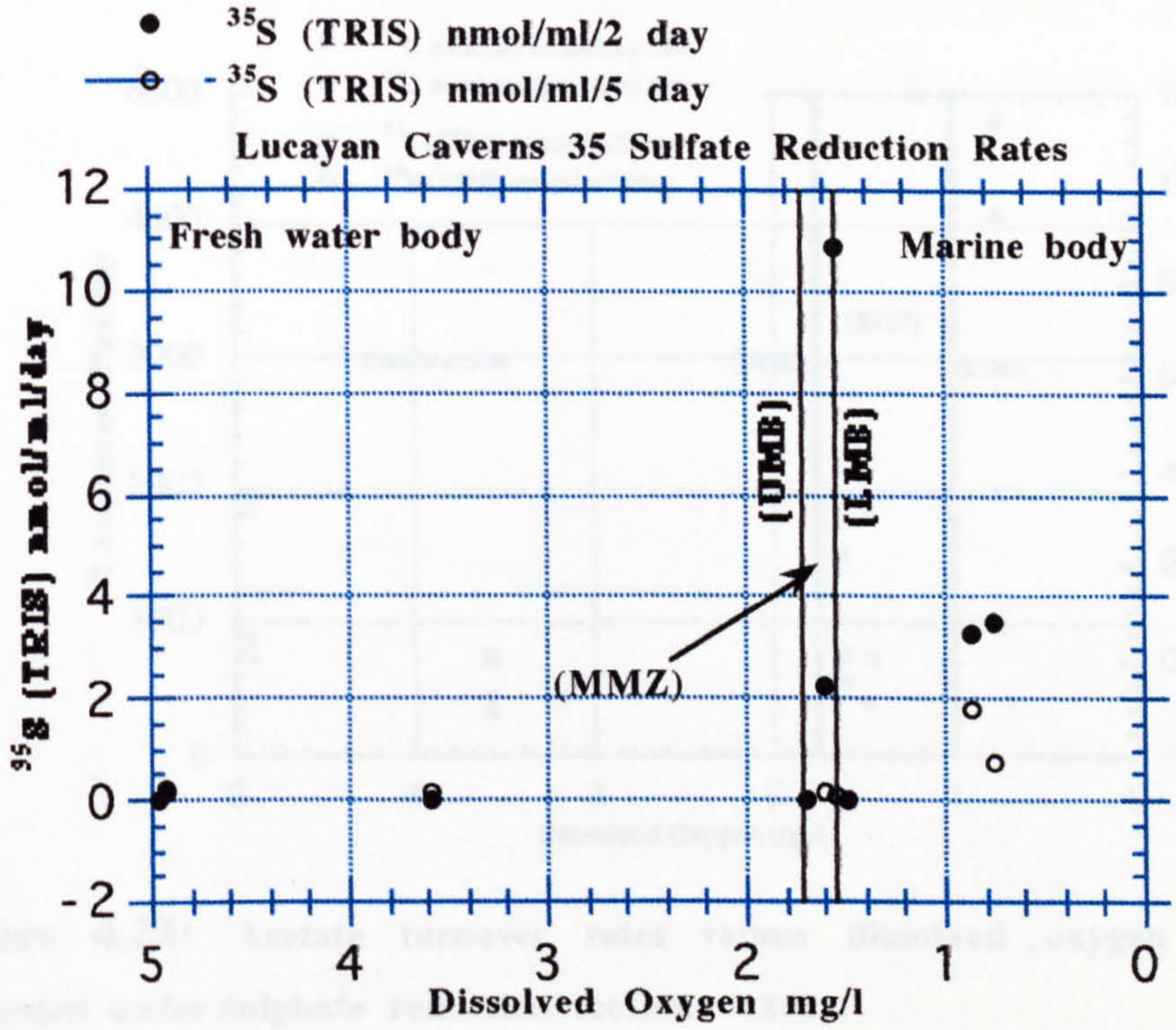


Figure 4.22: ^{35}S Sulphate plotted against dissolved oxygen mg/l (Lucayan Caverns)

sulphate reduction did occur at very low oxygen concentrations, mostly within the MMZ and the marine body; in the fresh water body there was virtually no activity. Chemical sulphide analysis using the modified Cline Assay were negative for both the radiolabeled ^{35}S samples and unincubated samples that were preserved with 20% acetate solution. This demonstrates that there are only very low amounts of sulphide present and this is rapidly oxidized.

The ^{14}C acetate and ^{35}S sulphate results were plotted against mg/l dissolved oxygen (Figure 4.23). Very little activity again occurred within the fresh water body, however, just below the UMB, rates began to increase and continued to do so well below the UMB with maximum measured activity within the near marine waters (LMB) where oxygen levels were at their lowest.

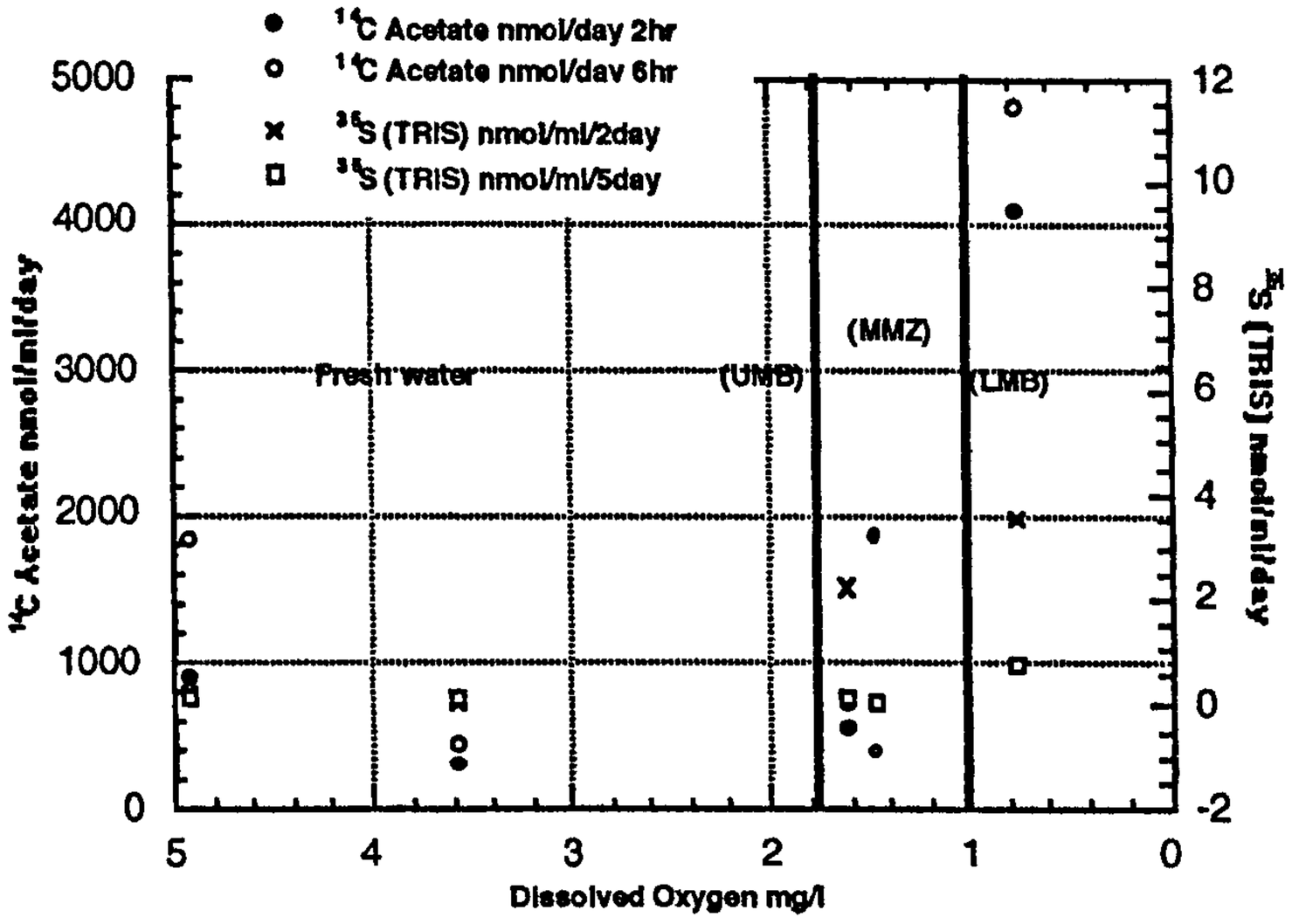


Figure 4.23: **Acetate turnover rates versus dissolved oxygen (described and presented under sulphate reduction section 4:2:3).**

4:3 Bacterial Counts

4:3:1 Media Plate results

Following 24 hour incubations of 100 ml of filtered water, all media plates had 100% growth cover, some growth even occurred up the sides of the plates. The objective was to try and count individual colonies so the water volume was decreased to 10 ml. However, this was still to much and eventually the volume of water was reduced to 1 ml which in some cases was still to much. Colonies grew in all different colours (white, light yellow, green, orange, brown, black and beige). The objective of trying to grow bacteria on media plates was to determine if bacteria were present within the cave systems and how easily they would grow.

4:3:1 Total Bacterial Counts

Bacterial cell numbers ranged from the minimum count of 21,203 cells / ml at 17 m, to a maximum cell count of 216,023 /ml at 15 m (Figure 4.24). Bacterial cell counts within the fresh water body averaged at 27,773 cells (± 0.04 n=3), MMZ, 168,667 cells/ml, (± 0.07 n=3) and 126,896 cells/ml (± 0.06 n=3) in the marine zone. Marked

bacterial populations, surprisingly, did not appear to respond to the density interface at the UMB at 14 m with a marked sharp change compared to the sudden changes that have been observed geochemically; bacterial numbers just continue to increase linearly straight through the UMB.

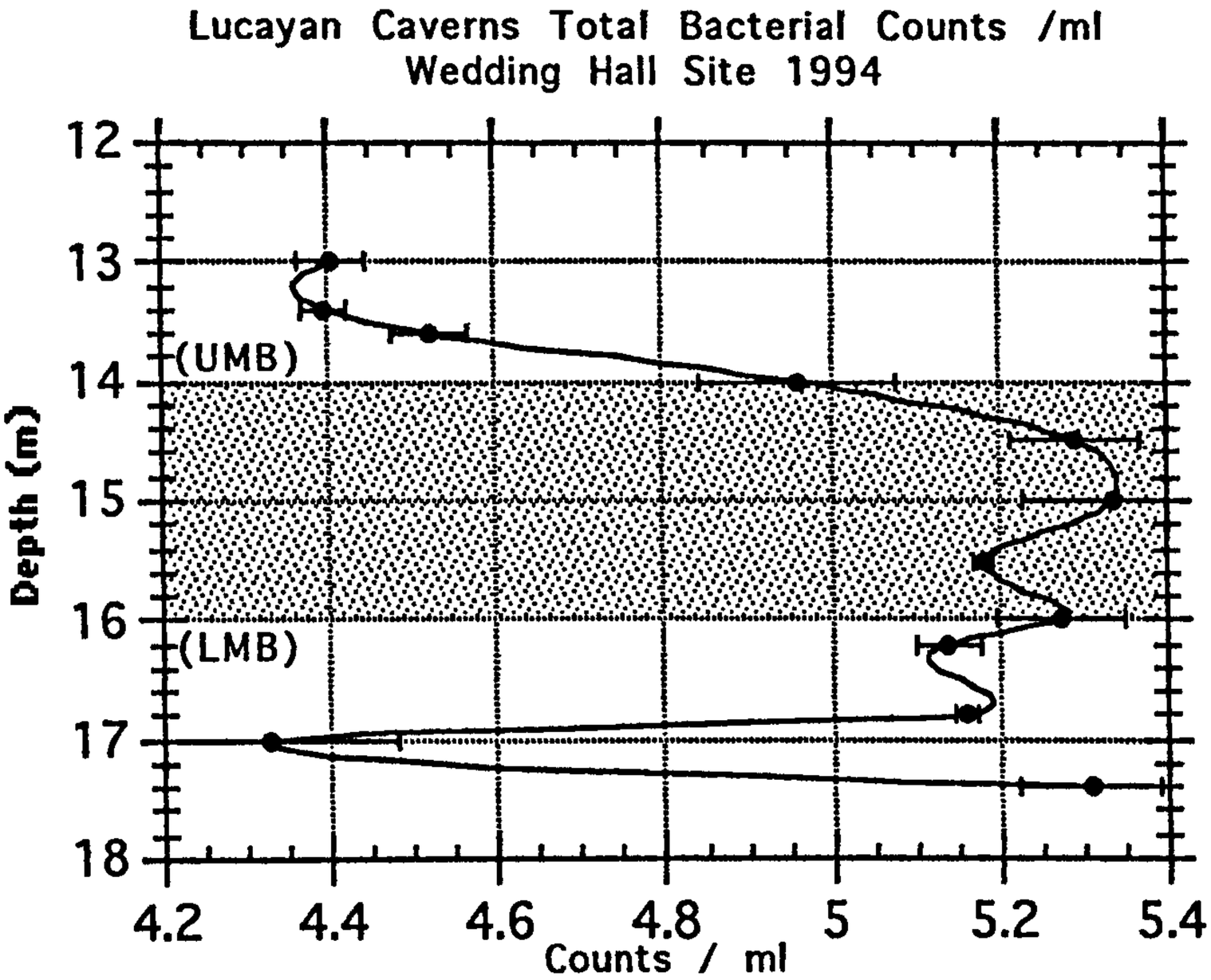


Figure 4.24: Total bacterial counts within the Lucayan Caverns sample site (error=n=3)

Bacterial cell numbers drop from 188,278 / ml ±0.07 n=3) at 16.8 m to 21,203 cells (±0.15 n=3) at 17 m. It appears that the MMZ is the favored location within the water column for bacteria. Total counts include dividing cells, cells on particles, and off particles. The deepest sample at 17.4 m is approximatey 12 cm above the floor. A possible explanation for the higher cell count in this sample is that a small amount of sediment may have been drawn into the sample upon collection.

4:3:2 Dividing Cells

Dividing cells are described in Figure 4.25 as a percentage of the total bacterial population count. The range of dividing cells was 0.8 % (± 0.03% n=3) as the minimum value at 16.8 m, to 15.2 % (± 0.19% n=3) at 14.5 m. The average was ca 3 % in the fresh water body, 6% in the MMZ, and within the marine body, 5%. These finding shows that within the MMZ

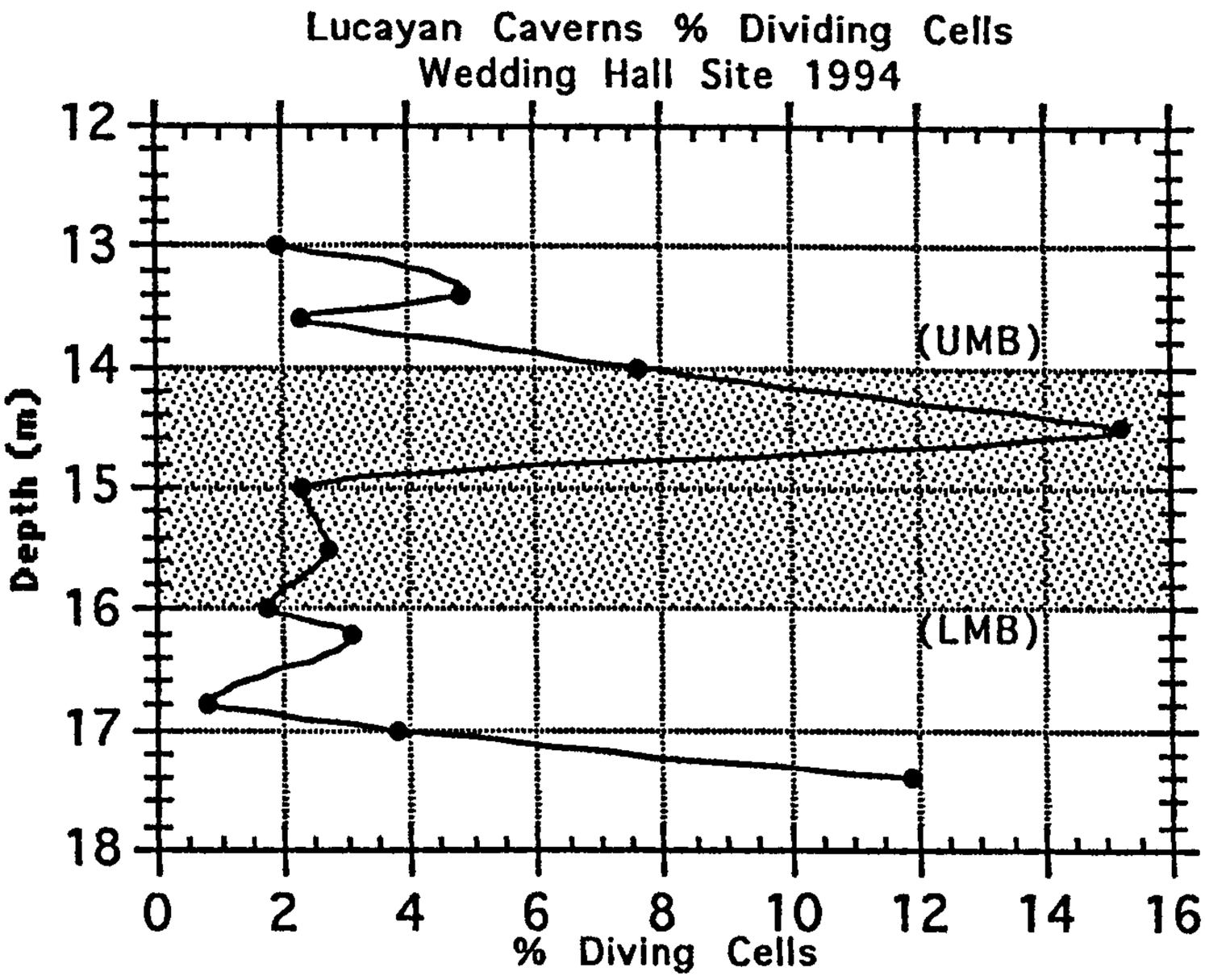


Figure 4.25: Graph showing percent bacterial cells dividing within the water column of the Lucayan Cavern sample site.

bacteria are dividing at a higher rate than anywhere else within the water column. The UMB and the LMB do not appear to have any influence on dividing cells. The proportion of dividing cells does increase through the UMB as well as in the total bacterial population.

4:3:3 Bacterial Cells on Particles

Bacterial counts on particles ranged from a minimum count of 0 counts/ml at 15 m, to 450 cells/ml at 17 m (Figure 4.26). Gradually, cell numbers increase with depth; fresh water average count is 166 cells/ml; 568 cells/ml within the MMZ, and in the marine body only 249 cells/ml.

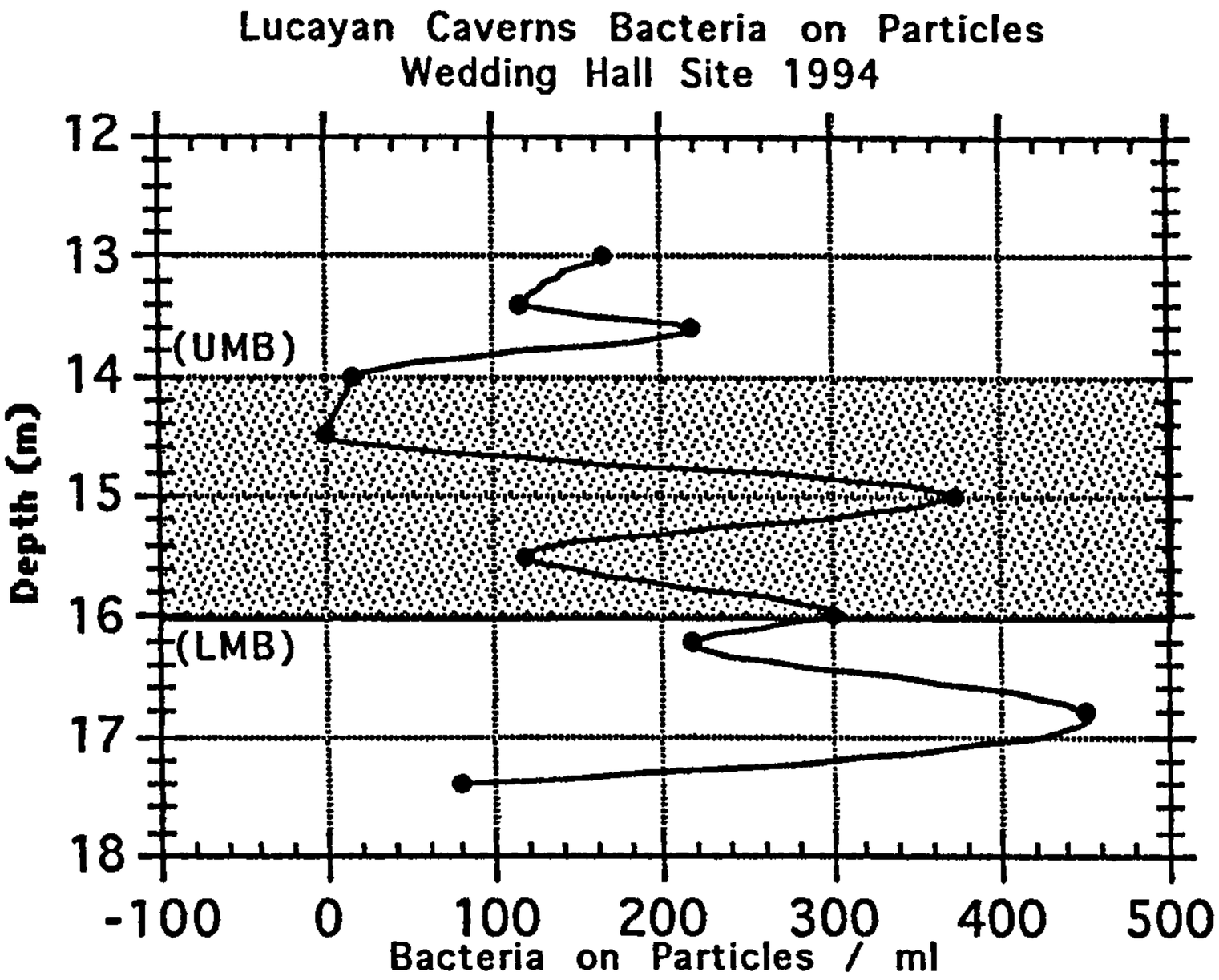


Figure 4.26: Bacteria-on-particle counts from the Lucayan Caverns water column site. (n=3)

The MMZ conditions do not seem to have any influence on bacteria on particle cells numbers. Cell numbers are very variable throughout the water column.

4:3:4 Bacteria Cells off Particle Counts

Bacteria-off-particle cell numbers are variable throughout the water column (Figure 4.27). The general trend is that numbers are decreasing with depth. The range is between 192 cells/ml at 16.8 m to the maximum cell count of 368 at 14 m for the UMB. The average number of bacteria within the fresh water body is 321 cells/ml, 317 cells/ml and for the MMZ, while 267 cells/ml were found in the marine body.

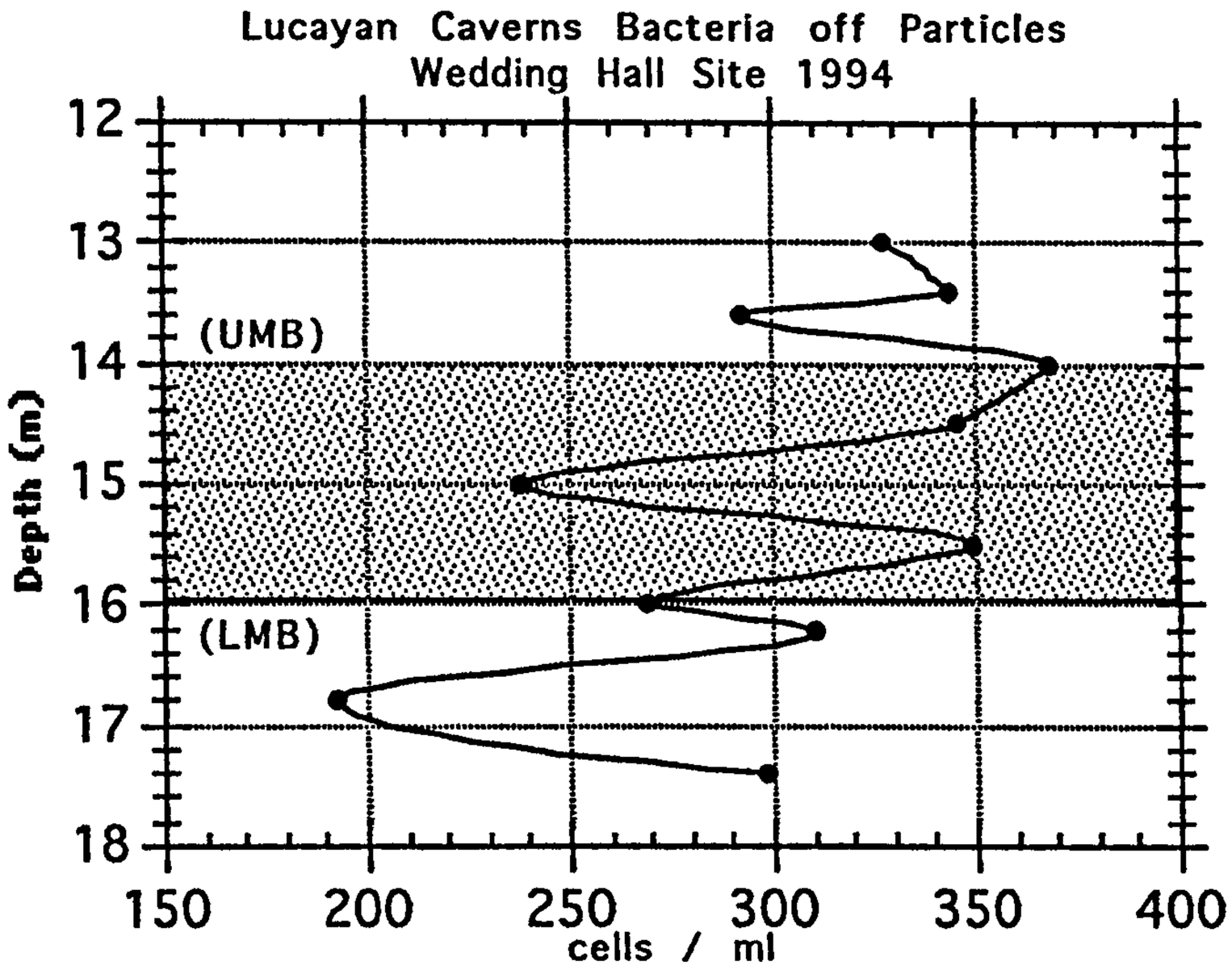


Figure 4.27: Bacterial counts off particles in samples from Lucayan Caverns.

Bacterial populations vary thoughout the sample site and cells do not appear to respond to the density step at the UMB or the LMB.

4:4 Geochemical Techniques

4:4:1 Amino Acid Analysis

The "mung material" collected off the cave floor and ceiling is a proteinaceous substance based on the amino acid results (Figure 4.28). The most often observed family of amino acids found within this sample are the "nonpolar" structures. These are glycine, alanine, valine, leucine, isoleucine, methionine, phenylalanine and proline; although proline is listed as an amino acid by some (Stryer, 1981). These amino acids, which are sometimes referred to as aliphatic (Stryer, 1981) interact more favorably with each other and with nonpolar compounds. These units are important for stabilizing the folded conformations of proteins (Jones, 1994). Hydrophobic proteins are likely to be embedded in the lipid-rich cytoplasmic membranes.

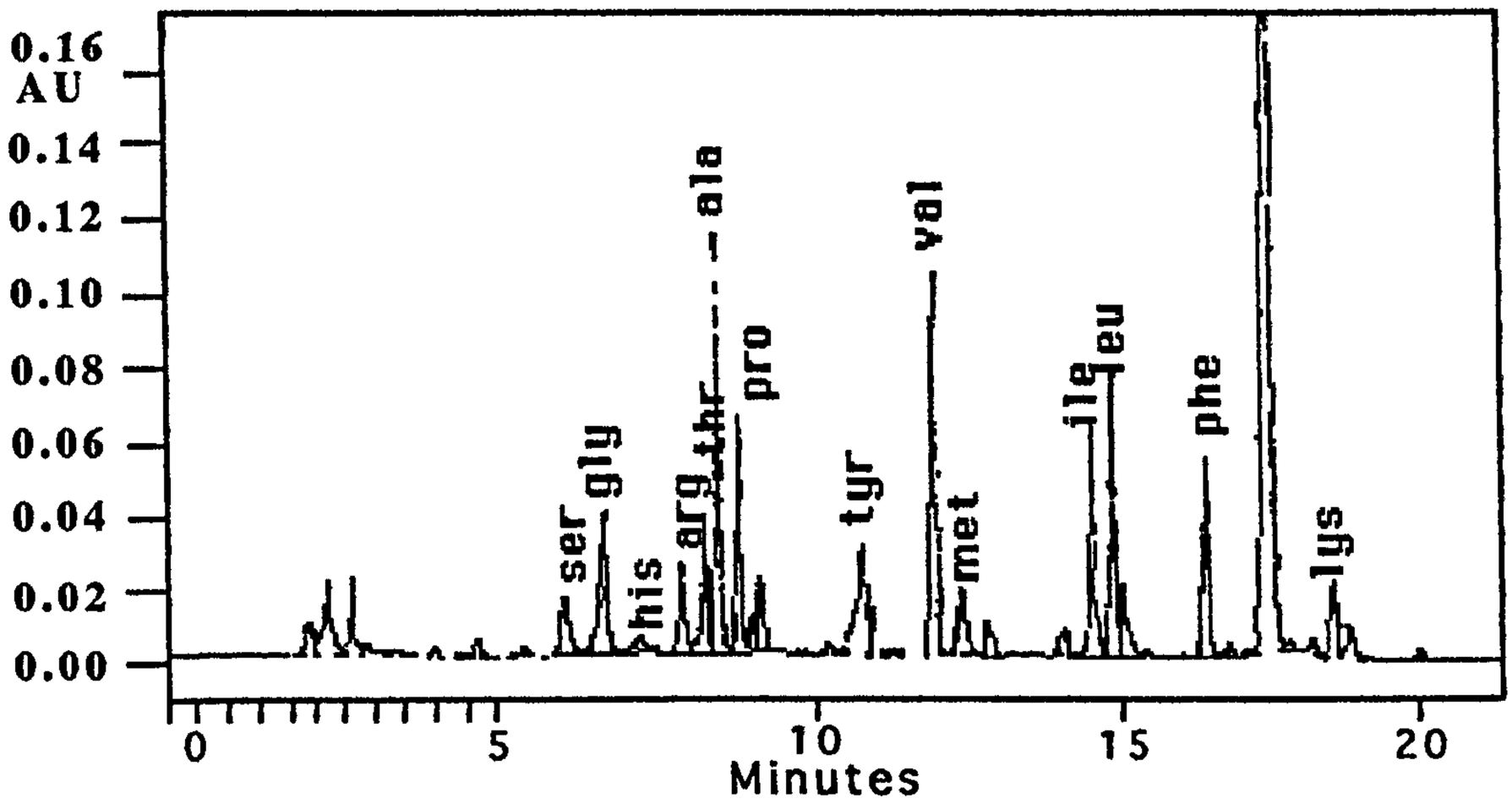


Figure 4.28: Chromatogram showing the amino acid composition of the "mung" material found within Lucayan Caverns

Of the seven known nonionizable polar amino acids, three were found within the sample: serine, threonine, and tyrosine (Figure 4.29). Serine and threonine contain aliphatic hydroxyl groups. Tyrosine is one of three common aromatic amino acids. There are two members of the ionizable acidic family which have acidic groups in the side chains, these are glutamic acid and aspartic acid. These two were low but discernable within the mung sample. All three of the ionizable basic amino acids were represented in this sample, i.e., lysine, arginine, and histidine. Lysine and arginine are positively charged whereas histidine is positively charged or neutral, depending on its local environment. Tryptophan is destroyed during acid hydrolysis and serine and threonine are reduced. The presence of branched chain amino acids and lysine suggests that the protein is bacterial in nature and the absence of amino acids in only hydrolyed samples leaves no doubt that the amino acids were derived from proteins present in the "mung" sample. Considering the fact that the mung was washed and pelleted by centifugation suggest further that the protein was not loosely associated with the material.

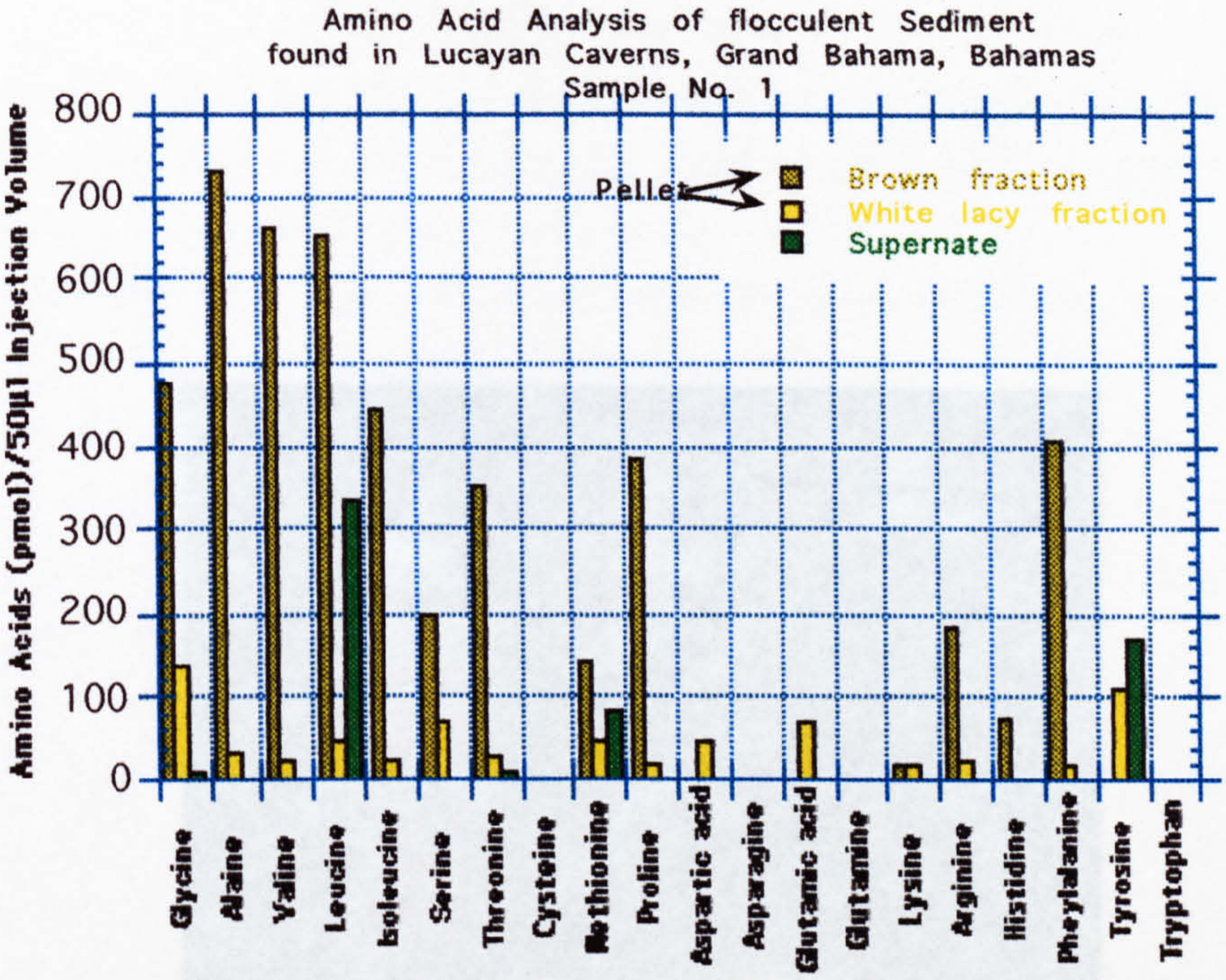


Figure 4.29: Bar graph representing the concentrations of the amino acids within different fractions of the samples.

4:4:2 Description of Sediment in Plexiglas Core

Three cores were recovered from the cave floor at the back of the Lucayan cave system. As soon as the cores were out of the water they were checked for sulfide odor, but none was detected. The sediment in all three cores was densely packed except for one core which had what appeared to be an air pocket. Of the three cores, only one was of a solid bright orange colour (Figure 4.30 Photo). The other two cores had between the red sediments thick layers of snow white sediment. In one core the white layer constituted about 1/4 of the core and 3/4 of the third core. At the very top of the cores was a crust approximately 2 mm in thickness. This crust was disturbed during the recovery process. Directly beneath the crust was a 4 to 5 cm section of soft sediment very similar to the proteinaceous bacterial floc (described earlier). Following the soft material was a 1 cm section which had multiple bands of less than 1 mm thickness, black and brown colours sandwiched between orange sediment.

After this banded layer, the sediment appears very homogeneous except for a few cracks which may have been caused by the recovery process. In the cores which

Figure 4.30: **Photo showing the colouration of the mud cores collected within the Lucayan Caverns site.**

had the white homogeneous unit, small black spots, the size of a pin head, could be seen. In some areas of the white deposit the black spots were patchy and dense and not so numerous in other areas. Between large sections of the white deposits were thin bands of the red clay approximately 2 mm thick.

Bacterial counts were difficult to obtain because the only way to dissolve this sediment was with concentrated HCl treatment. Bacterial cells did not survive the concentrated HCl. Sonication and a dilute solution of HCl were tried to see if bacterial cells were present. Results showed the presence of a few bacterial cells, however, without effectively removing the iron, an accurate bacterial count could not be obtained.

4:4:3 X-Ray Defraction

With the XRD results is was posssible to identify the red mud in the cores as "Lepidocrocite", an orthorhombic, biaxial crystalline material (Figure 4.31 and Table 4.2). It is more commonly referred to as "hydros iron oxide". Composition and structure of lepidocrocite is analogous to boehmite and consists of cubic close-packed O atoms with Fe^{3+} in octahedral interstices between the layers of O (Nesse, 1986). This sample, however, is a fibrous form which is more commonly found.

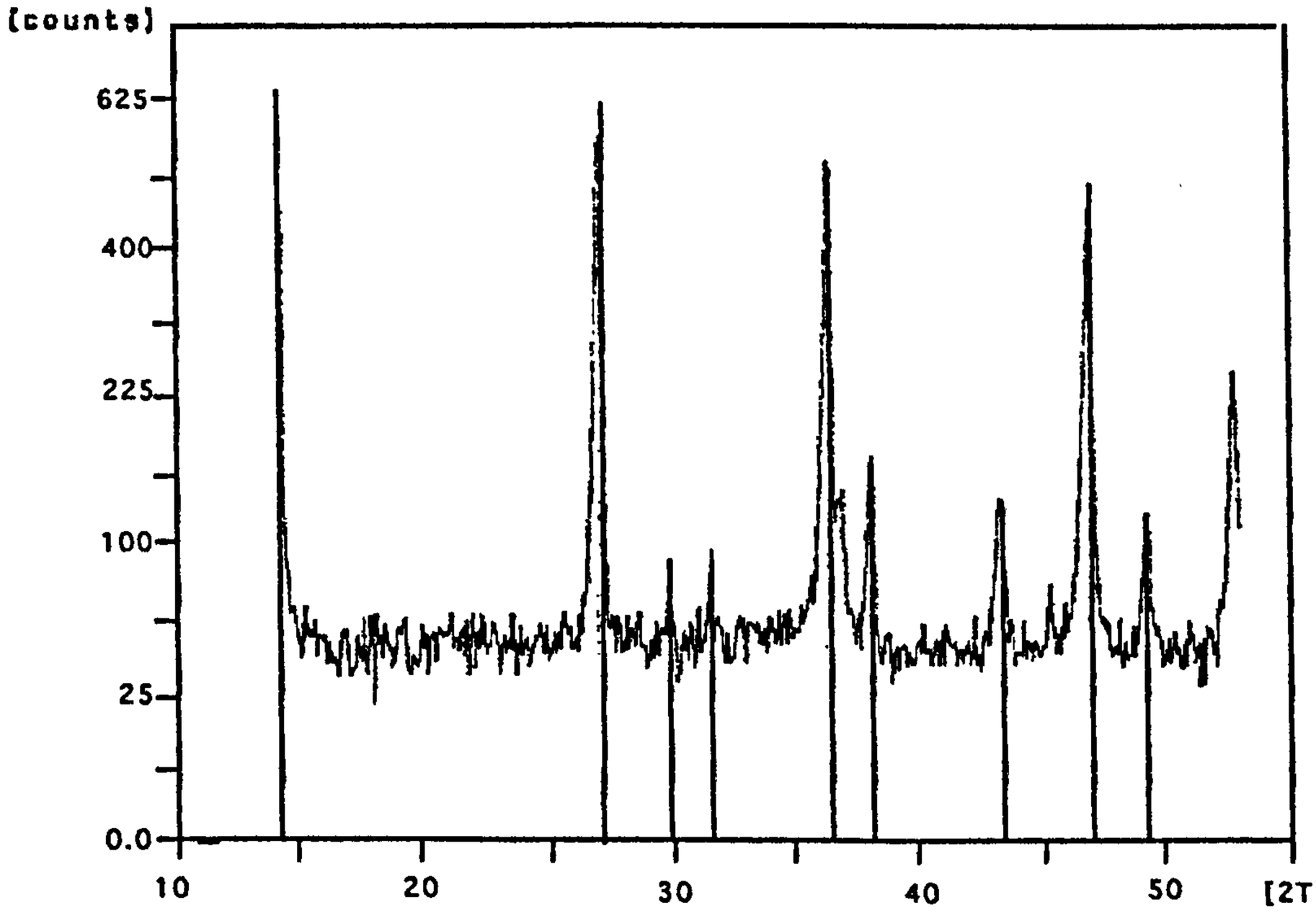


Figure 4.31: XRD sample identification graph for mud sample from the Lucayan Caverns site on Grand Bahama.

Table 4.2 XRD information identifying mud sample from Lucayan Caverns as lepidocrocite (Mineral Powder Diffraction File Data Book, 1980)

8-98 occurs typically under oxidizing condition Lepidocrocite

d	6.26	3.29	2.47	6.26	∂ FeO(OH)Iron Oxide HydroxideColour red to red brown	Dehydrates to ∂-Fe O_2 3soluble in HCL (conc)Orange streak
I/I I	100	90	80	100		
Results from Sample from Lucayan Caverns						
d	6.23	3.28	2.46	6.23	found in suboxic to oxic conditionColour red to red brownsoluble in HCl (conc)	Orange streak
I/I I	100	90	80	100		

Lepidocrocite has a hardness of 5 and a density of 4.09. This mineral will only dissolve in concentrated HCl and nitric acid with a high acid volume to sample ratio. The mineral is brown to red in hand samples and stains bright orange streaks. Skin contact causes orange staining which cannot be washed away immediately. Lepidocrocite is formed by the weathering or hydrothermal alteration of Fe-bearing minerals. It is a common constituent of lateritic soils, along with goethite and hematite, and may be found in the supergene zone of hydrothermal sulfide deposits (Nesse, 1986). The white fraction of the core is suspect of being micrite mud. It was not analyzed for this study. The varying units of lepidocrocite and white mud within the core (Figure 4.30) present interesting questions concerning the history of passed depositional sequences within the cave, however, that was not the focus of this project and only the iron deposit was examined for its potential association with microbial activity within the cave environment.

4:4:4 Carbon, Hydrogen, Nitrogen & Sulphur (CHN&S) on Mud Sediment Samples

Three samples measured positive for sulfur and two of the three same samples measured positive for organic carbon (Figure 4.32). Samples containing organic carbon along with sulfur came from the same sample; deep section of the cores ;10 to 12 cm. The shallower sample at 5 cm contained sulfur but no organic carbon.

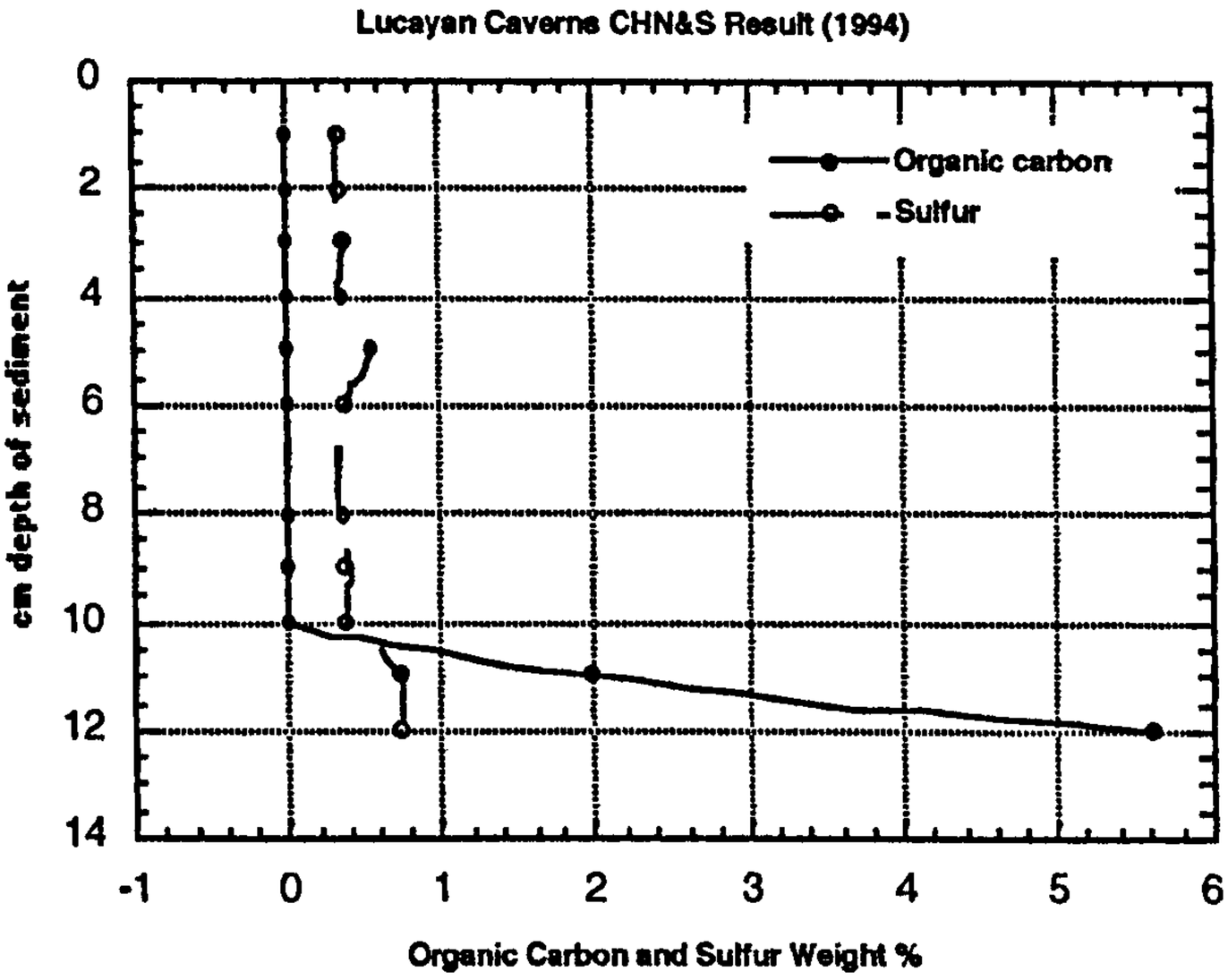


Figure 4.32: Consecutive amounts of organic carbon and sulphur of a sectioned mud core.

4:4:5 Scanning Electron Micrograph (SEM) of Sediment Samples

A lepidocrocite section four cm from the top of the core was examined by scanning electron micrograph. SEM photographs at 40 μm, although not highly informative, show a crumbly substrate of various sizes. At 10 μm the particles begin to take on a fuzzy powdered appearance. At 4μm, however, it could be seen that the minerals had a fibrous form also orthorhombic shapes. The mineral was extremely difficult to observe at high magnification because of rough multi-surfaces. The gold sputter process was not successful in coating all surfaces, making viewing very difficult and, in higher magnification, impossible. The SEM photos reveal a fibrous morphological form not unusual for lepidocrocite.

4:5 Geology

4:5 1 Rocks: General Observation

Geological analyses have shown that caves in the Bahamas have formed almost exclusively in bioclastic deposits (Schwabe 1992, unpublished M.Sc. Thesis). With this in mind, wall rock samples were collected to characterize the deposits in which

these blue holes had formed. At the entrances of some of the study sites, paleo-reefs could be observed and it is not uncommon to find whole walls composed of nothing but conch shells (*Strombus gigus*) and cement. In some passages sand dollars can be seen sticking out of the walls. Petrological examinations of the wall rocks helped to characterize the rock in which these caves formed.

4:5:2 Thin Section Petrology

All 7 samples collected from the front of the cave and from the back of the cave are bioclastic. A sample from Avalanche Alley (Figure 4.3) is characterized as an allochem and is the only site where this type of rock was found within this system thus far.

The bioclastic rocks (refer to Table 1.1b) are composed of foraminifera, gastropods, serpulid worm tests, ostracod tests, and bivalve shells. What is interesting, however, is that there was no sign of any algae. This is unusual because most samples collected from caves located above sea level almost all had some form of algae, most common was the coralline algae and *Halimeda.*

A feature which did vary among samples was porosity. Samples collected above the mixing zone, both at the front of the cave and the back, had porosity values between 10 to 15%. Rock samples collected within the mixing zone had porosity values of 20 to 30 %. Samples from below the mixing zone, moving into marine water conditions, had 1 to 3%, and some had no porosity at all and were well cemented; findings similar to those in the dry caves (Schwabe, et al., 1993).

Samples collected from within the mixing zone had very few composite grains but quite a few micrite envelopes. Porosity was achieved by dissolution of the grains, leaving micrite cement rims. The cement history of the grains indicates that at some time the composition of the circulating groundwaters changed. Samples show a history of marine, aragonitic circumgranular cements and fresh water spar cements. Samples below the mixing zone showed no hint of such a cementing history. This is due to what is referred to as over-printing. Composite grains in samples collected below the mixing zone have been altered to such a point that they cannot, in most cases, be recognized. Most of the samples are composed almost exclusively of microcrystalline cements.

Samples from Paul's Palace Owl's Hole, Grand Bahama

4:6 Owl's Hole Geochemistry

4:6:1 Salinity

The fresh water body within this system is approximately 6 to 7 m thick but could be thicker. Uncertainty of its exact thickness is based on the fact that part of the lens, as in the Lucayan site, is found within the ceiling rock (Figure 4.33). Again using the biological limits of fresh water at 0.5 g/l salinity, the fresh water lens is just outside of this value at 0.75 g/l. The boundary of the fresh water lens, for the purpose of this study is defined as begining at 11 m.

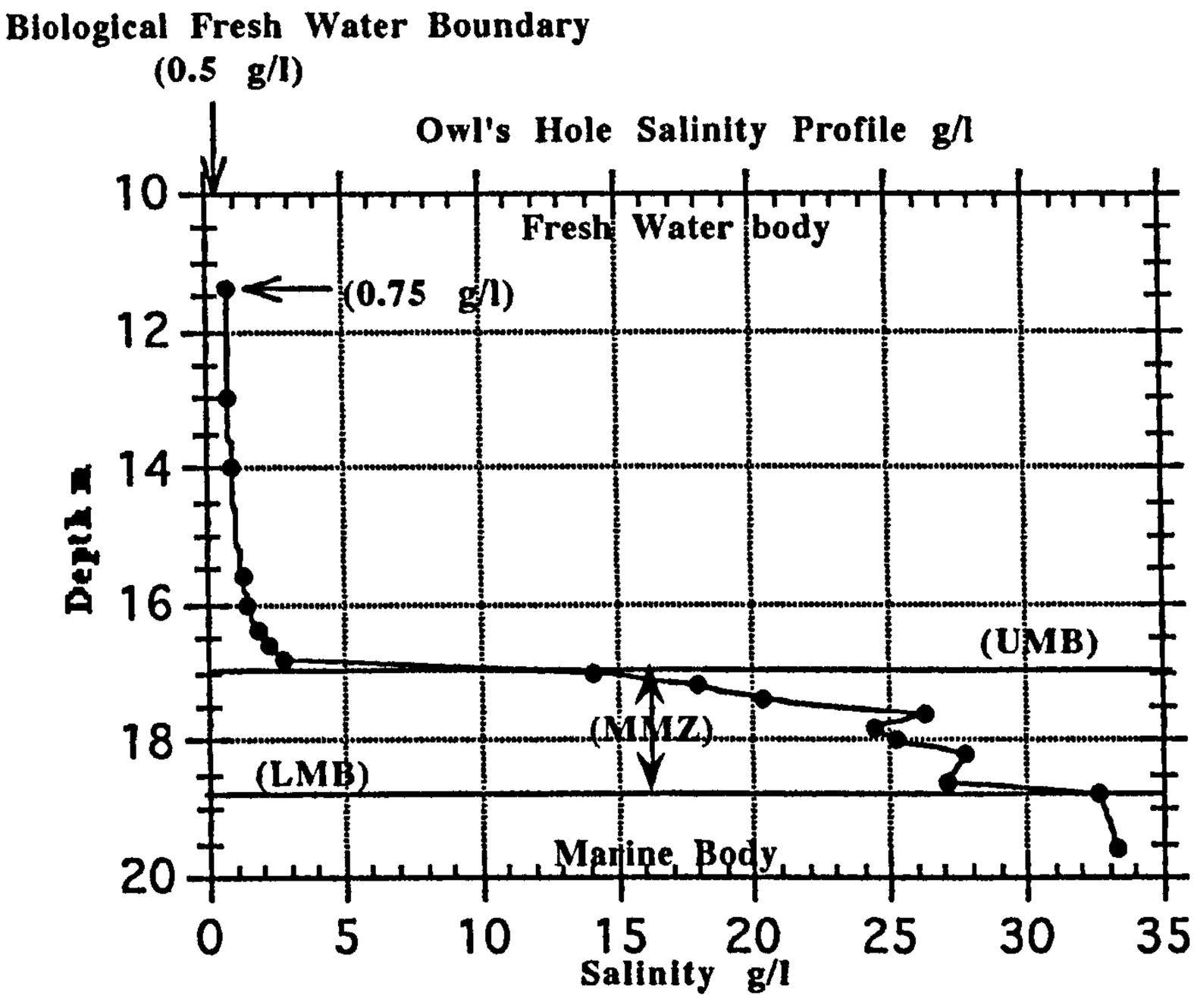


Figure 4.33: Owl's Hole salinity profile

In Lucayan Caverns, the fresh water body extended from 11 m to the top of the UMB at 16.8 m. At Owl's Hole the UMB is at 17 m and the MMZ, almost two metres thick, ends at the LMB at 18.8 m. Below 18.9 m there is the marine body. The salinity range was from 0.75 g/l at 11 m to 33.25 g/l at 19.6 m. The average salinity of the fresh water body was 1.52 g/l, MMZ, 24 g/l, and of the marine body, 33.25 g/l.

Salinity increase within the MMZ is broadly linear whereas in the fresh water body the increase is exponential towards the UMB. As at the Lucayan site, the UMB

and the LMB are both sharp chemical and visual transition zones and just as pronounced as in the Lucayan Cavern.

Looking at the hydrolab salinity profile for comparison (Figure 4.34), the 1998 results confirms that the UMB is at 15 m and the LMB is 18 m or possibly at 19.6 m, if one does not segregate the MMZ by the lower salinity step found between 18 m and 19 m. The LMB is 0.8 m deeper than the 1994 recording of the LMB, a factor which may be explained by tidal fluctuation within the cave system.

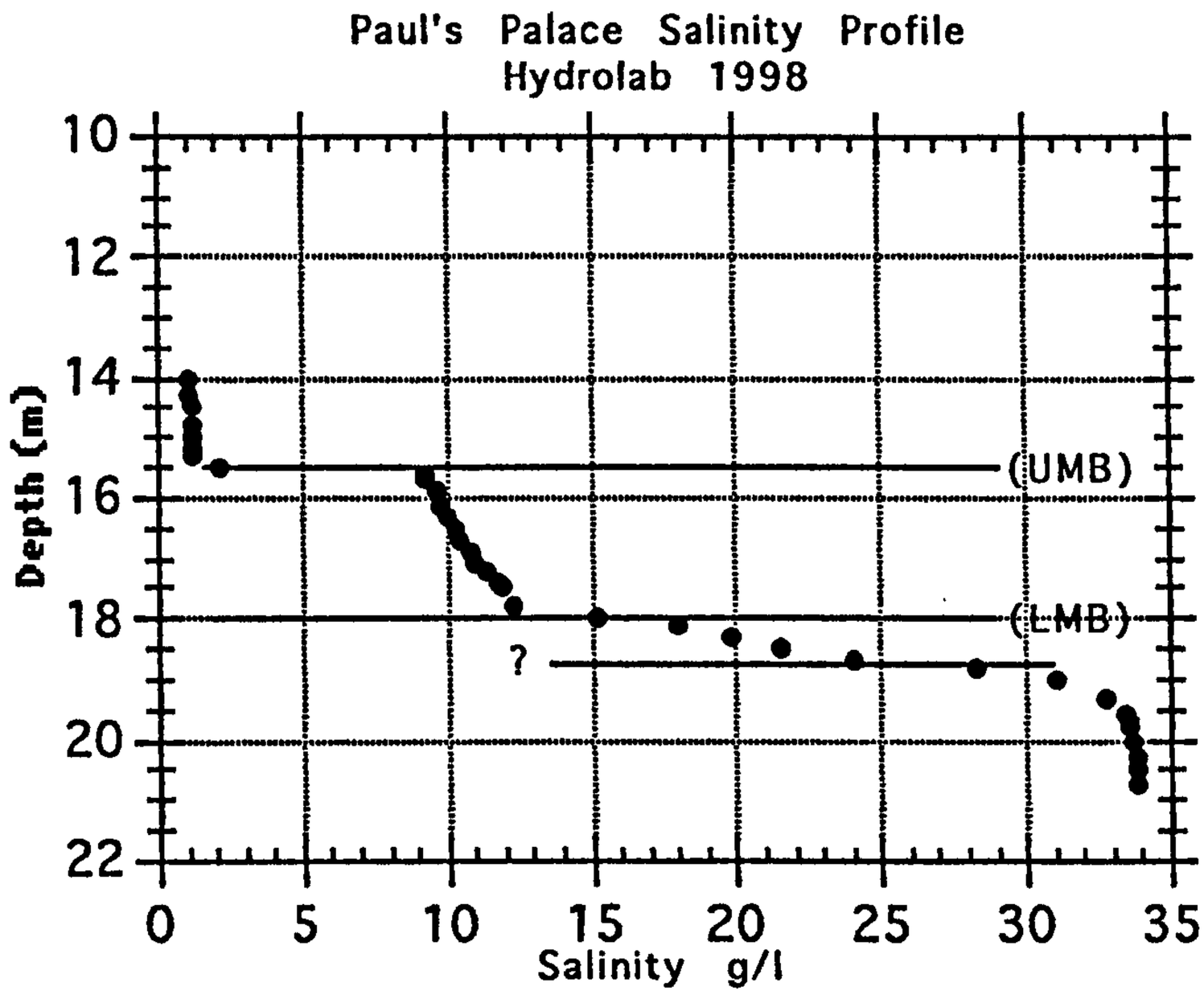


Figure 4.34: Hydrolab salinity profile for Paul's Palace within the Owl's Hole Cave system.

4:6:2 Temperature

Temperatures ranged between 21.8^0 C at 19.6 m, to 26.9^0 C at 16 m, a difference of 5.1^0 C (Figure 4.35). From 13 m to 16 m, the temperature increases linearly and in deeper samples the temperature varies dramatically. Interestingly however, each time the temperature increases, it does so linearly. Maximum temperature changes occurred at 16 m, just above the UMB, and at the UMB at 17 m. Following that, the temperature is again elevated slightly just before passing through the LMB at 18.7 m.

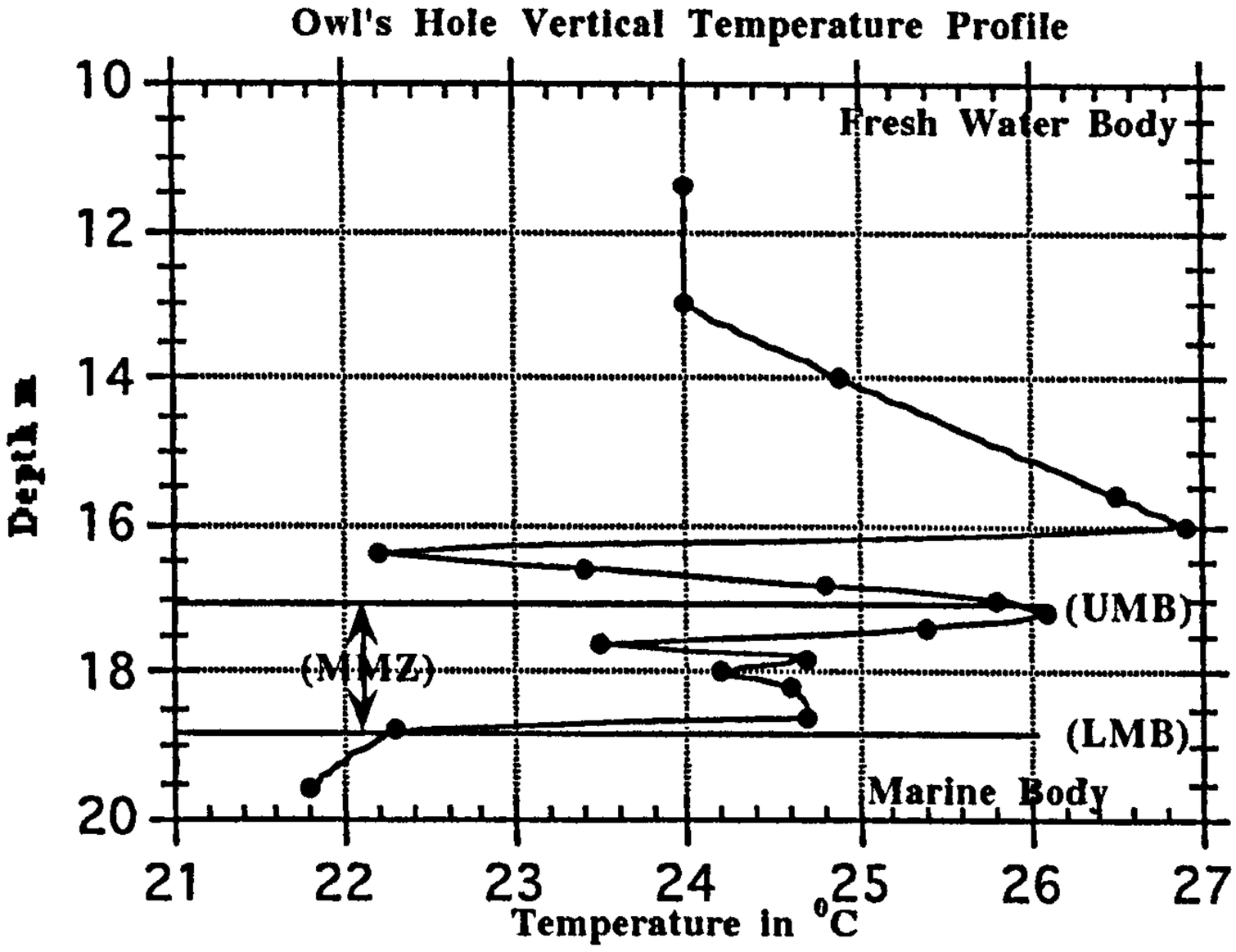


Figure 4.35: Temperature profile for the vertical water column in Owl's Hole

From this depth onward, the temperature, although only two data points are available, appears to continue to cool. The average temperature for the fresh water body is 24.7°C, 24.8°C for the MMZ, and for the marine body, 22.0°C. Again, as seen at the Lucayan site, the MMZ appears to be the warmest body of water within the water column at this site.

The hydrolab results (Figure 4.36) demonstrate that temperature conditions are quite different from the 1994 results. Although temperature results do reflect changes across mixing zone boundaries, overall, the water column is cooling with depth. Here

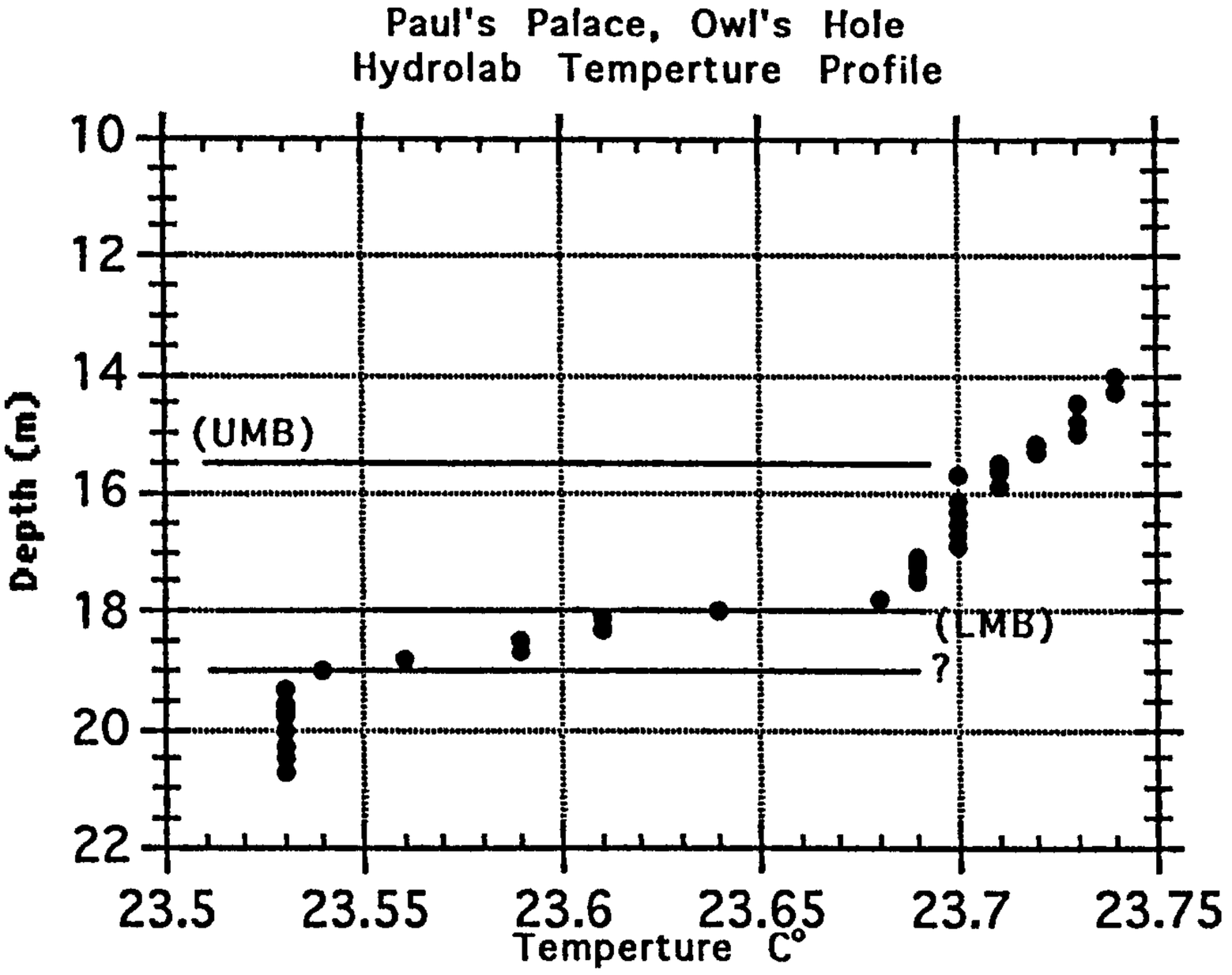


Figure 4.36: Hydrolab temperature profile for Paul's Palace, Owl's Hole

again, temperature measurements were taken *in situ* whereas the 94/96 results were attended to immediately following the dive.

4:6:3 pH Hydrogen-Ion Activity

The fresh water body was slightly alkaline in comparison with the MMZ, with pH values ranging from pH 7.47 at 16 m in the fresher water to pH 7.2 at 18 m in the MMZ (Figure 4.37). Fluctuations of pH values within the water column may be in response to the different micro lamina flow layer which can visually be observed within the vertical water column. These fluctuations may also correspond to the temperature changes described earlier in section 4:6:2.

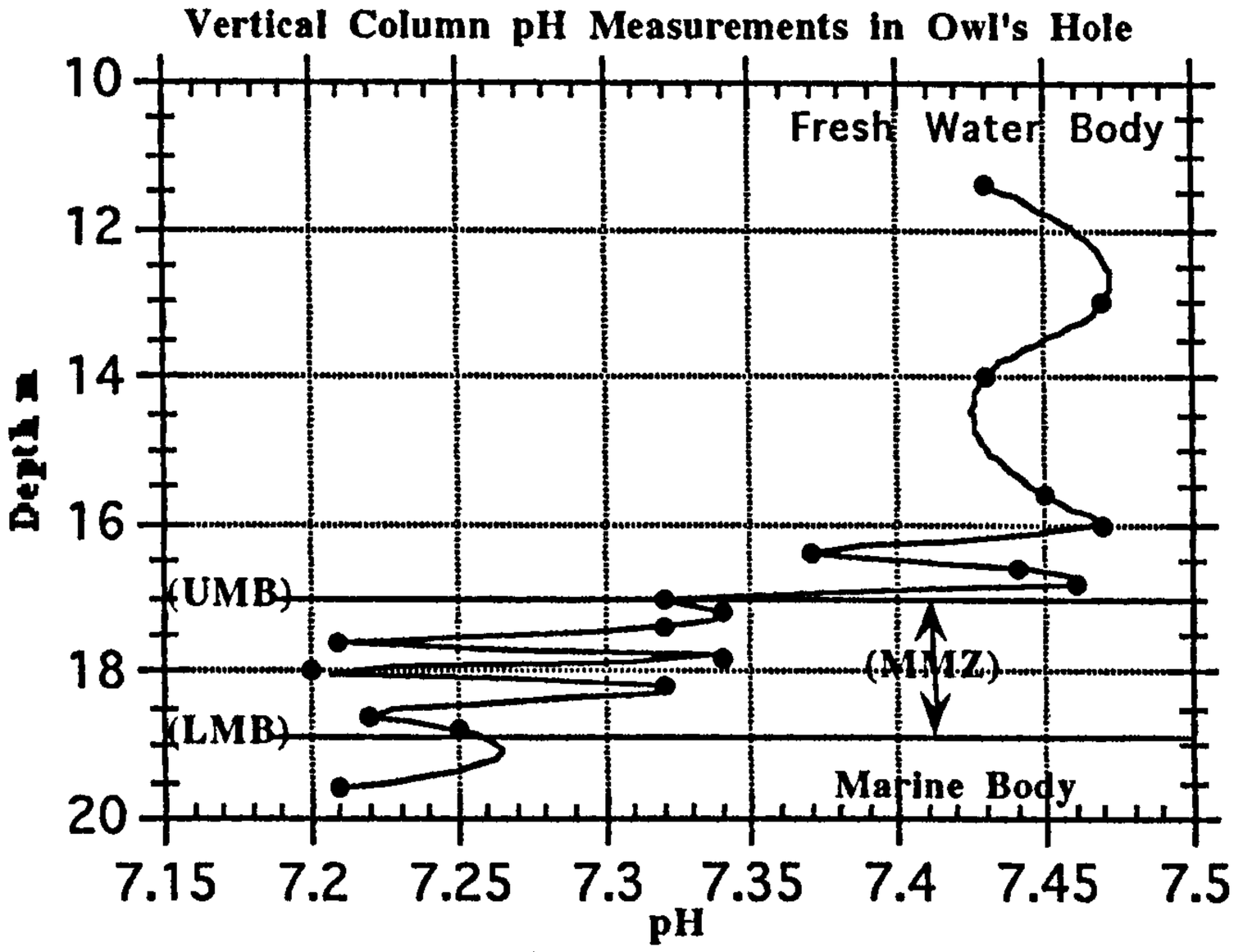


Figure 4.37: Owl's Hole vertical pH profile through the water column.

Again, as seen at the Lucayan Caverns site, although not as sharply defined, the MMZ is slightly more acidic than the fresh water body. The average for the fresh water body was pH 7.44, the MMZ, pH 7.3, and for the marine body, pH 7.21.

The hydrolab results (Figure 4.38) show that the more acidic portion of the water body is the body of water following the LMB. Overall, the pH value is decreasing from the UMB

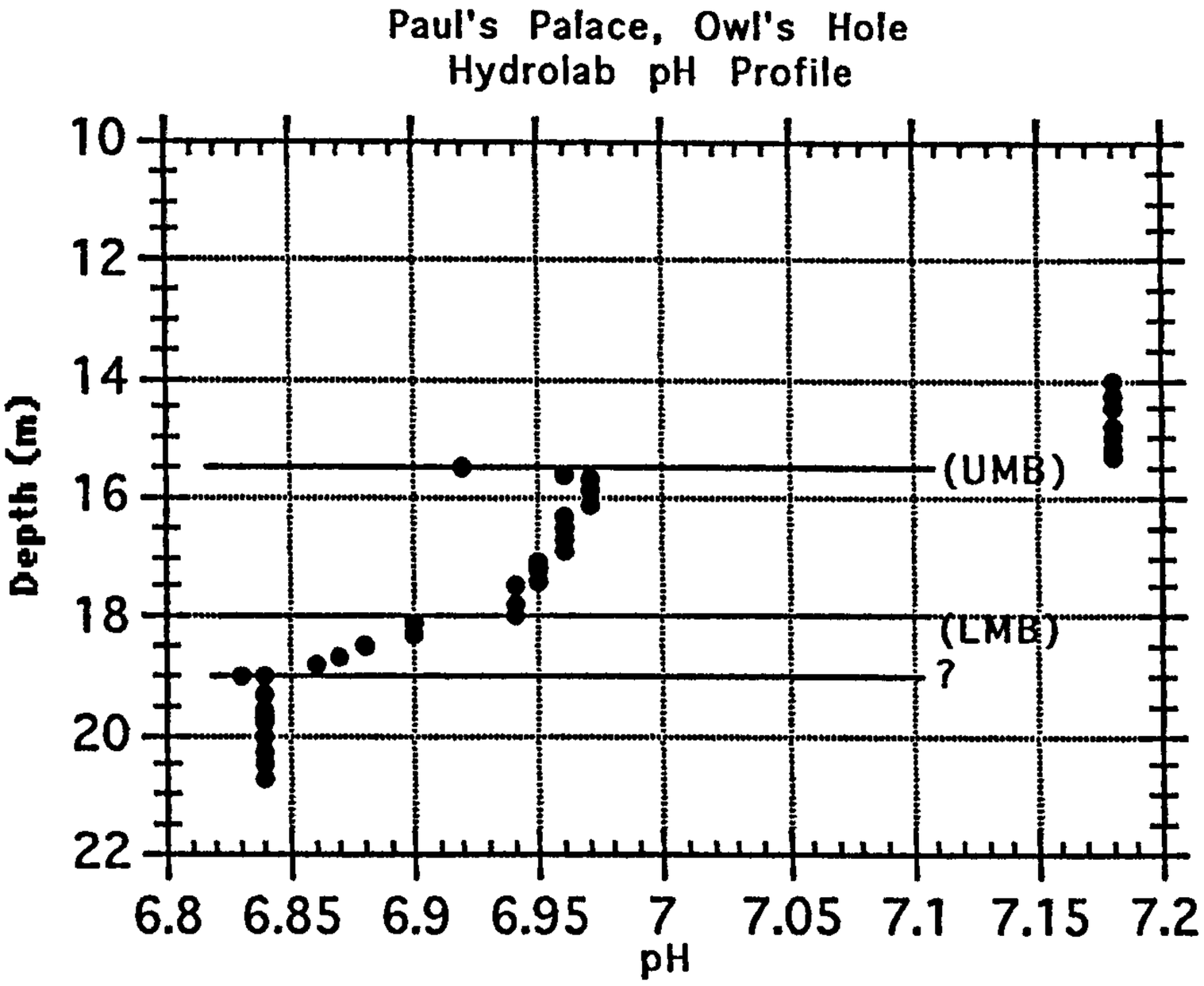


Figure 4.38: Paul's Palace, Owl's Hole hydrolab pH profile

throughout the MMZ to the LMB. The fresh water body has a very stable pH as well as the marine section of the water column. However, another interesting difference is that overall the lower section of the fresh water lens (15 m) is more acidic than the 1994 results demonstrate. For example, the 1994 pH reading for 15 m was at pH 7.43 units whereas in the 1998 results, the same depth had a pH measurement of pH 6.96. Basically, the region from the UMB well into the marine section is, for the 1998 results, much more acidic.

4:6:4 Alkalinity

Bicarbonate alkalinity decreases with depth as would be expected based on pH measurements and other geochemical parameters such as temperature and salinity (Figure 4.39). Alkalinity decreased exponentially throughout most of the water column.

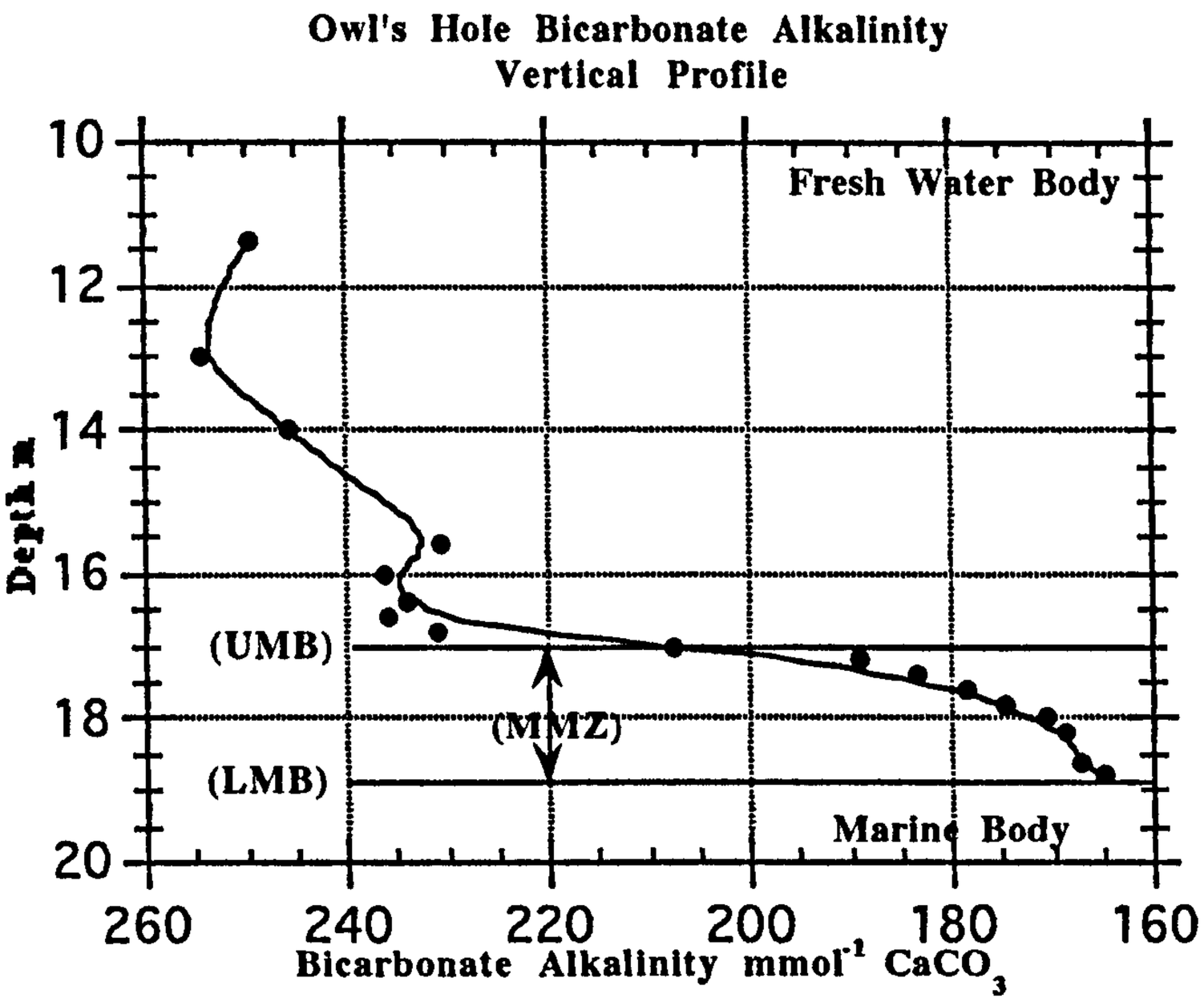


Figure 4.39: Bicarbonate alkalinity profile through the vertical water column in Owl's Hole

The average alkalinity for the fresh water body was 241.7 $mmol^{-1}$ $CaCO_3$ and for the MMZ, 178.8 $mmol^{-1}$; below the LMB no samples were collected. Alkalinity for Lucayan Cavern and Owl's Hole are very similar (refer to figure 4.12)

4:6:5 Dissolved Oxygen

Dissolved oxygen decreases relatively rapidly with depth (Figure 4.40). The 11.4 m depth sample contained 7.5 mg/l dissolved oxygen (DO); saturation for this water sample should be 8.3 mg/l DO. DO averages for the fresh water body was 4.60 mg/l, for the MMZ, 1.48 mg/l, and the marine body, 0.78 mg/l.

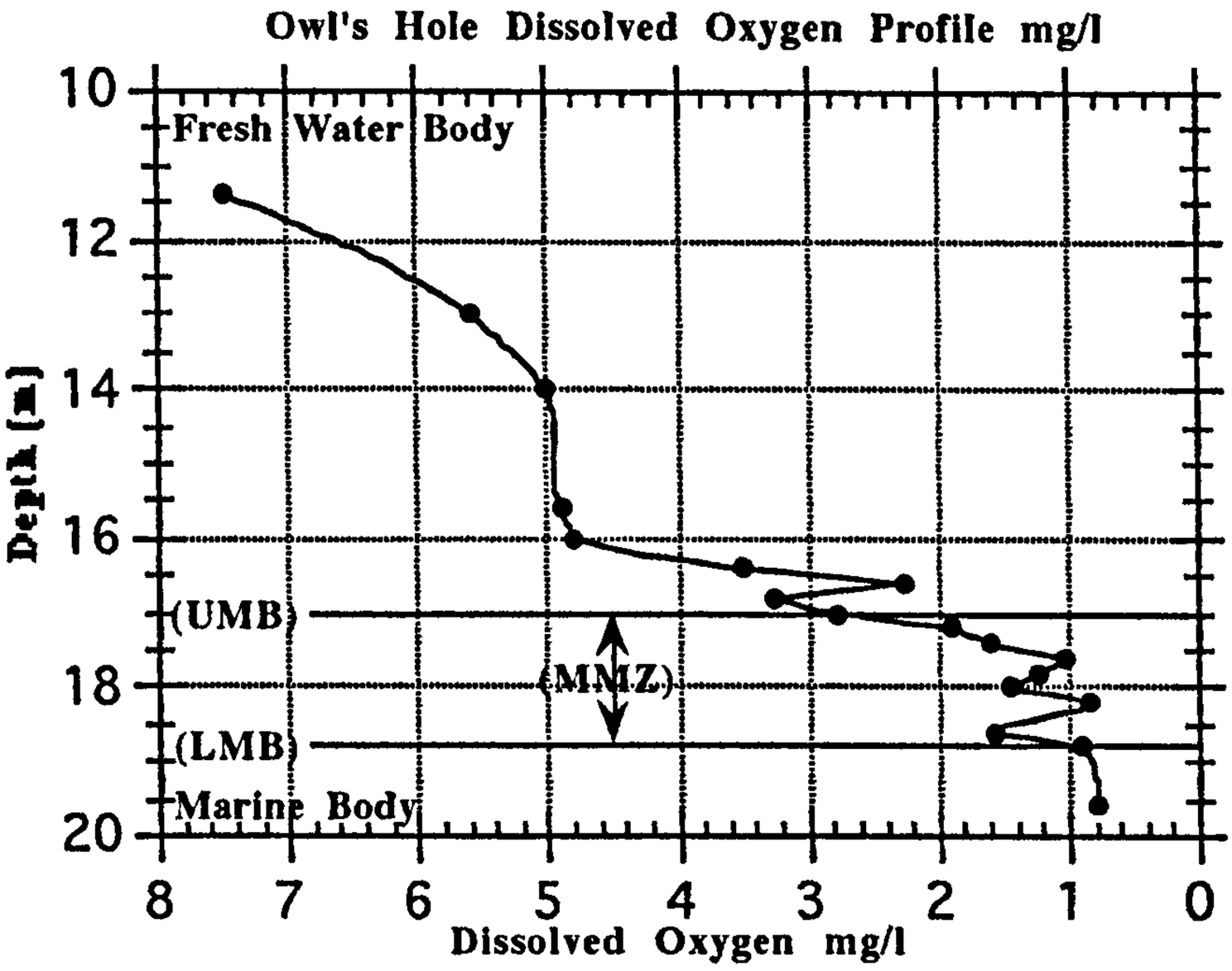


Figure 4.40: Vertical water column profile for dissolved oxygen (mg/l) in Owl's Hole

Oxygen concentrations decrease stepwise from the fresh water body towards the UMB, and between 14 m and 16 m, the DO changes very little. From 16 m, the decrease is exponential till 16.6 m when the decrease becomes variable through the UMB, through the MMZ, till the LMB is reached. From this depth it appears that DO values decrease more slowly again.

The fresh water sample at 11.4 m is undersaturated by 6.3%. The fresh water within Owl's Hole is significantly more oxygenated than Lucayan Caverns (refer to Figure 4.13) where the most shallow water sample collected at 13 m was already 40% undersaturated. The (UMB) in Owl's Hole is undersaturated by 79%, compared to Lucayan Caverns 65%. What is interesting is that the LMB in Owl's Hole is 90% undersaturated compared to 81.8% at Lucayan Caverns. A possible explanation is that the bacterial population is higher in the LMB in Owl's Hole, where heterotrophic bacterial activity is consuming more oxygen.

The hydrolab results (Figure 4.41) demonstrate the same decrease in DO values as seen in the 1994 results and overall the DO values are very similar. For example, at 15 m, the 1994 results showed that at this depth the DO value was 4.8 mg/l. In comparison, the hydrolab results show a similar value of 4.6 mg/l. The same trend

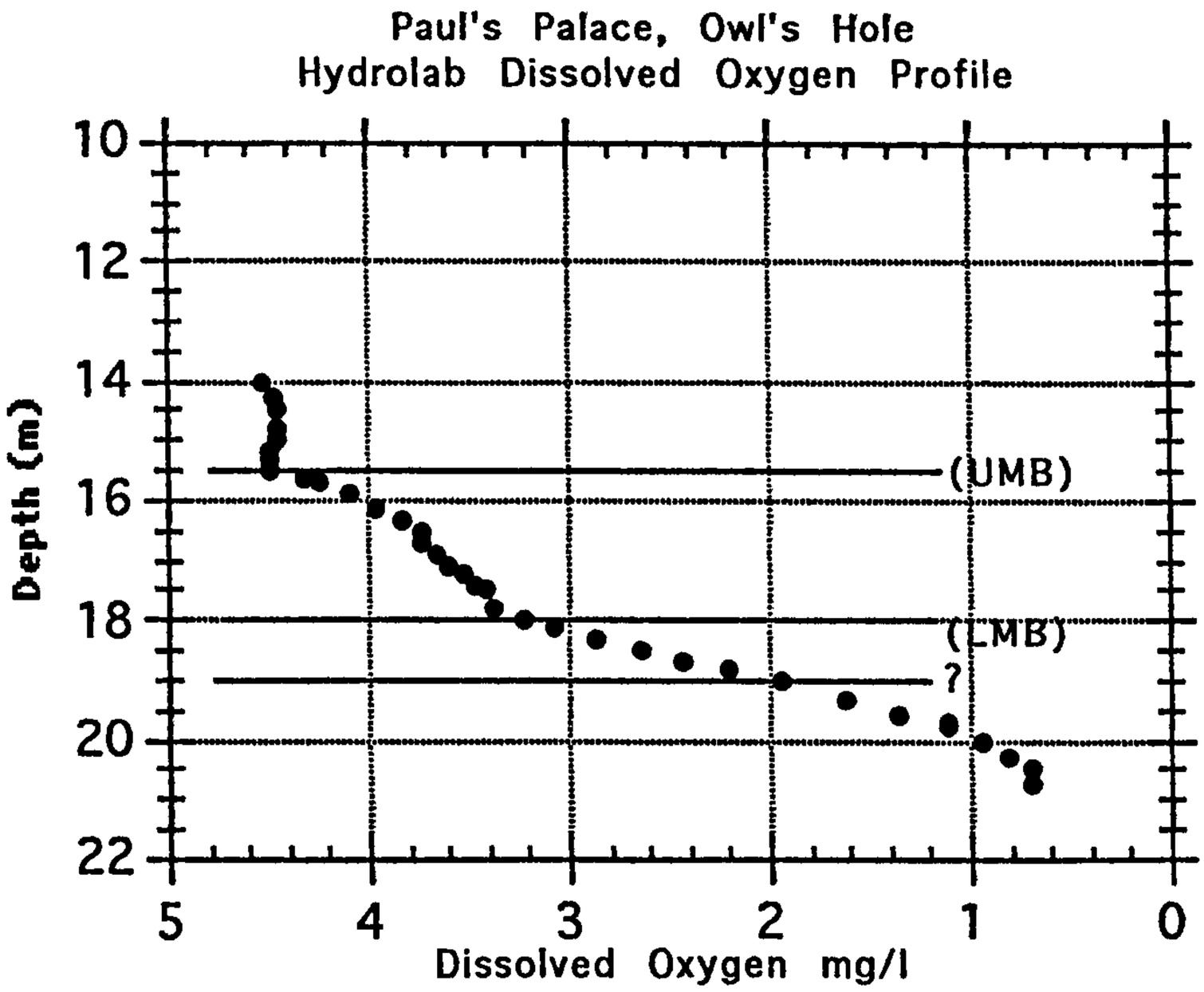


Figure 4.41: Paul's Palace, Owl's Hole hydrolab dissolved oxygen profile

can be observed at 18 m where the 1994 and the hydrolab results are identical with a result of 2.8 mg/l DO. Values at 19 m for both 1994 and the hydrolab are slightly different with consecutive values at 0.8 mg/l for 1994 and 1.8 mg/l for the hydrolab. Again it is possible that tidal variations may account for the slight differences.

In examining the multi-profile recovered from Owl's Hole (Figure 4.42), it is possible, as seen in the earlier Lucayan multi-profile (refer to Figure 4.16), to see changes over distance traveled into the cave system. Unlike Lucayan, the salinity (Figure 4.42 A), temperature (Figure 4.42 B) and for the most part, except for the 4th DO profile (Figure 4.42 C), the geochemical

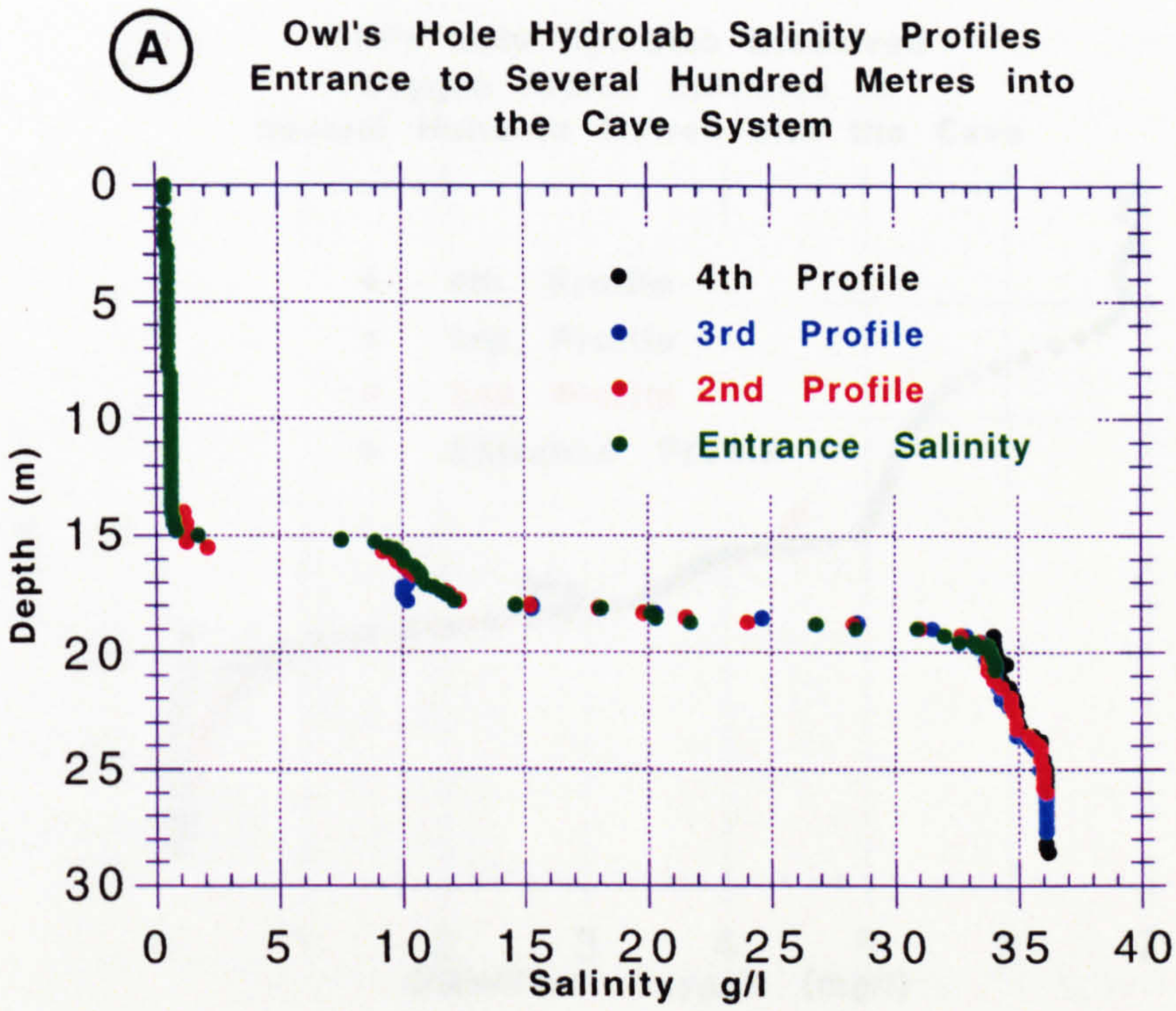


Figure 4.42 (A): Multi-hydrolab profiles, salinity from the entrance of Owl's Hole to several hundred metres into the cave system. The second profile is Paul's Palace.

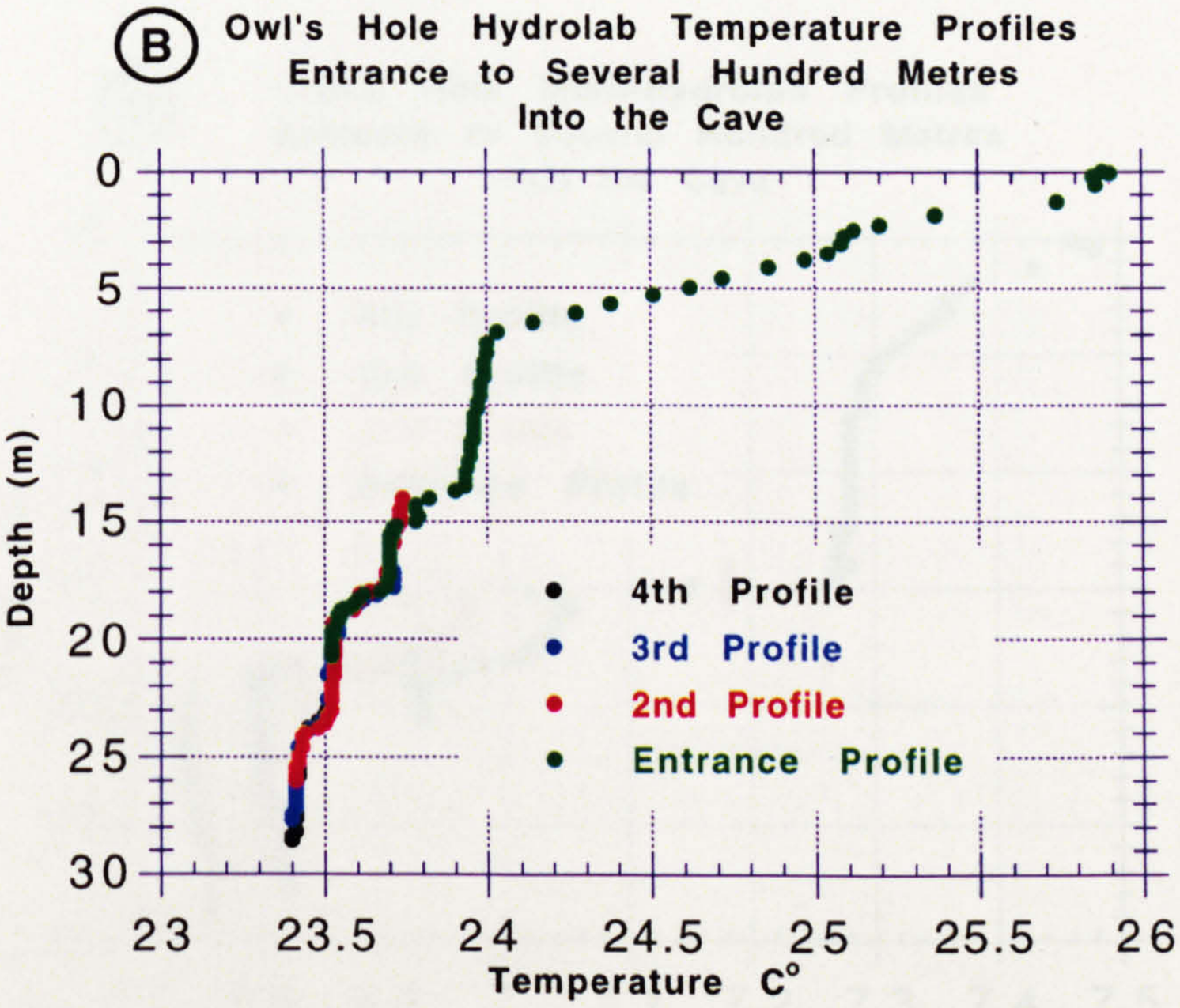


Figure 4.42 (B): Multi-hydrolab profiles, temperature from the entrance of Owl's Hole to several hundred metres into the cave system. The second profile is Paul's Palace.

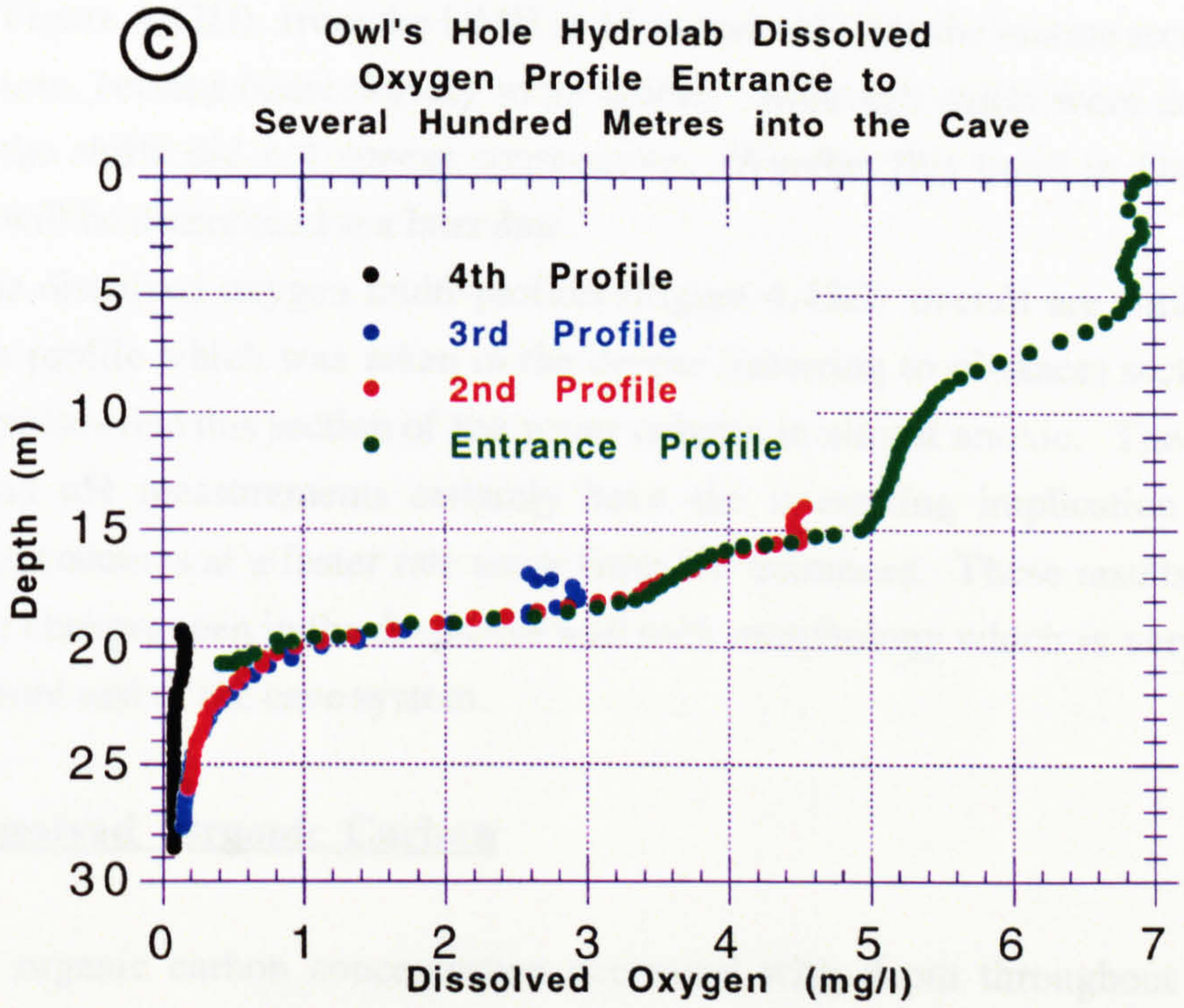


Figure 4.42 (C): Multi-hydrolab profiles, dissolved oxygen from the entrance of Owl's Hole to several hundred metres into the cave system. The second profile is Paul's Palace.

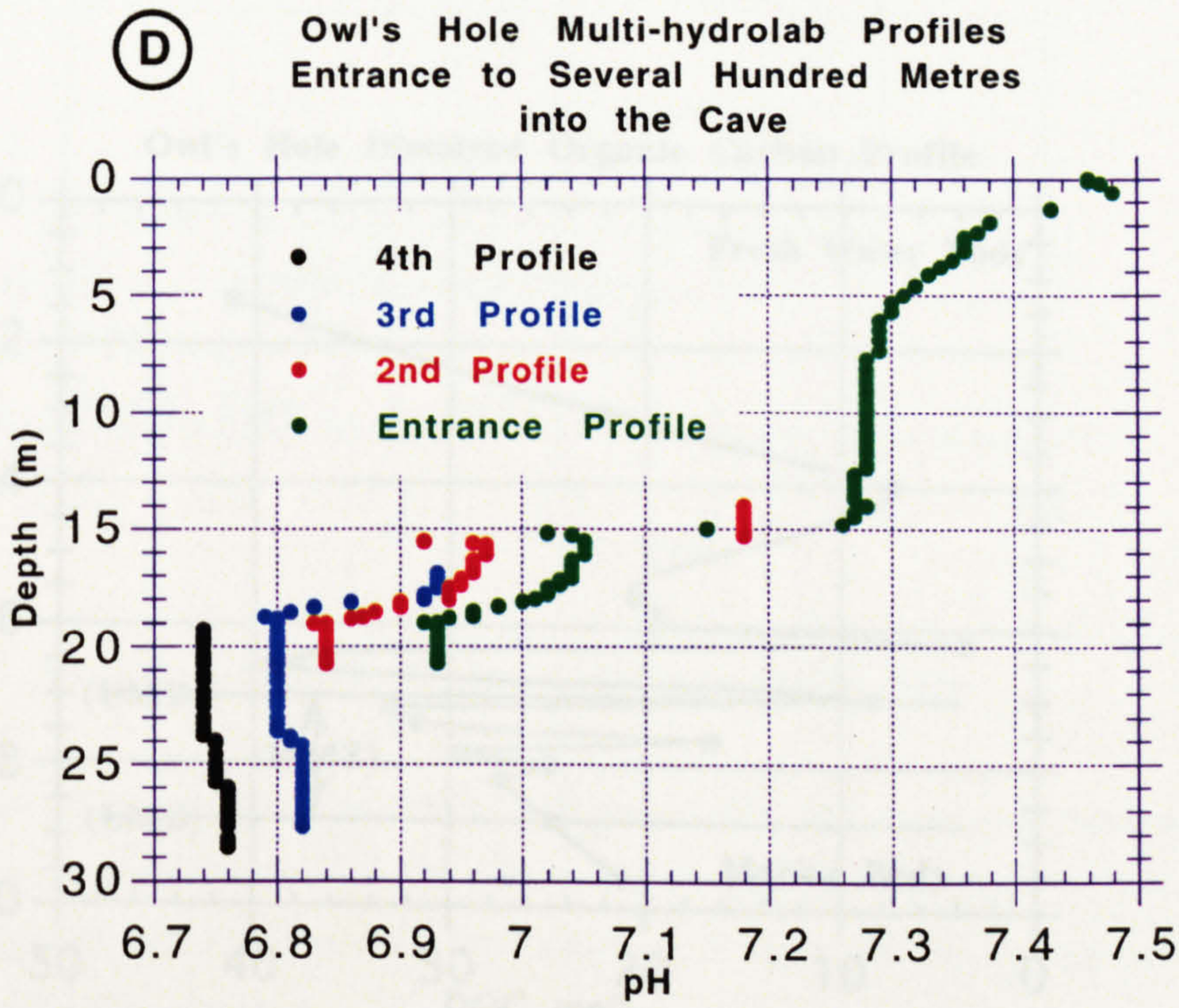


Figure 4.42 (D): Multi-hydrolab profiles, pH from the entrance of Owl's Hole to several hundred metres into the cave system. The second profile is Paul's Palace.

perameters remain unchanged over distance. The pH values within Owl's Hole however (Figure 4.42D), from the UMB at 15 m and well into the marine section of the water column, became consecutively more acidic. Although shifts were seen within Lucayan, the shifts did not appear consecutive. Whether this trend in Owl's Holes continues will be determined at a later date.

The dissolved oxygen multi-profiles (Figure 4.42C) overall are similar except for the 4th profile which was taken in the deeper (referring to distance) section of the cave. It appears that this section of the water column is almost anoxic. The combined oxygen and pH measurements certainly have the interesting implication that cave development occures at a faster rate away from the entrances. These results may also explain the changes seen in the deep cave wall rock morphology which is very different from the front end of the cave system.

4:6:6 Dissolved Organic Carbon

Dissolved organic carbon concentration decreased with depth throughout the fresh water body till 14 m. Deeper DOC was highly variable through the UMB and the uppermost MMZ until 18.2 m when, again, concentrations increased with depth exponentially (Figure 4.43) into the marine body. The DOC average was 3.64 mg/l at 16.2 m, for the MMZ, 24.5 mg/l, and 21.17 mg/l for the marine zone.

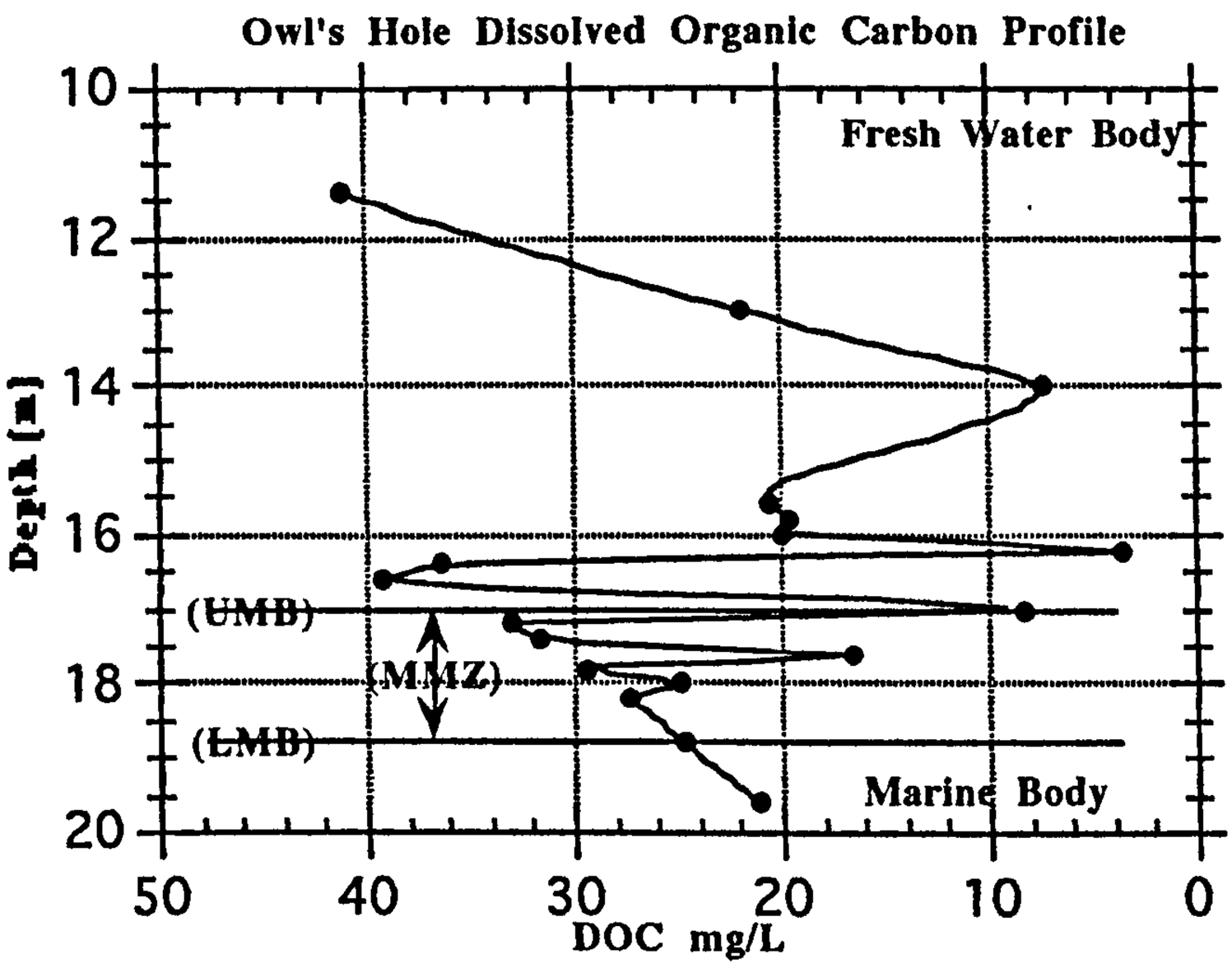


Figure 4.43: Vertical profile of dissolved organic carbon in Owl's Hole

The first sample had the highest concentration of DOC (42 mg/l) found within the vertical water. Again, variations within the deeper section of the water column may be in part due to micro-and macro-layered lamina flows and density gradients which can be seen as light brown banding throughout the water column. These bands are much more visible in the Owl's Hole site compared to the Lucayan Caverns site.

4:6:7 Acetate concentrations

Acetate concentrations within Owl's Hole fluctuate throughout the water column, similar to the Lucayan Caverns site, but overall concentrations are less within the vertical water column (Figure 4.44). The range of acetate within the Owl's Hole site was zero at 14 m, and the maximum concentration was 22.2 μM at 17.4 m.

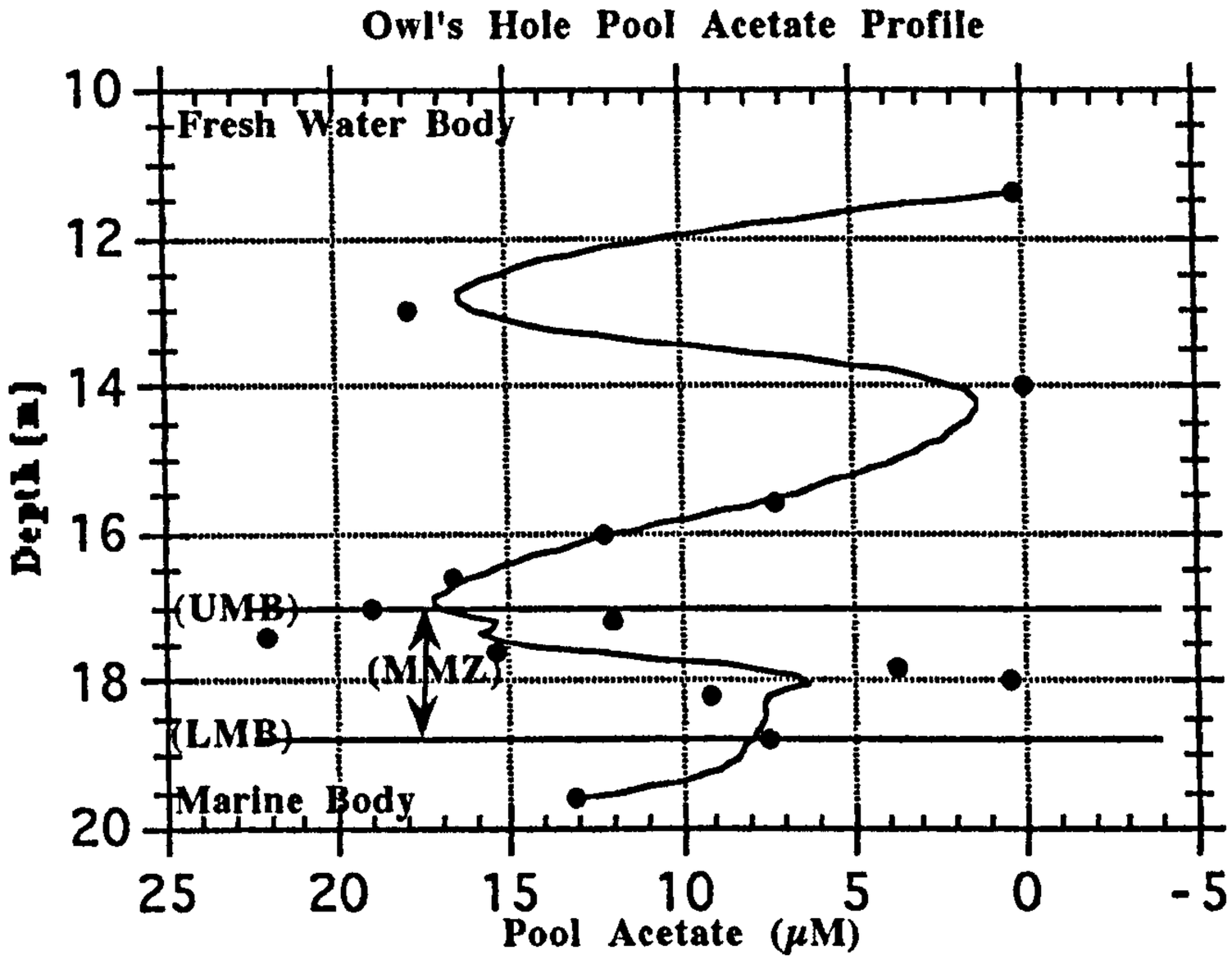


Figure 4.44: Vertical profile for pool acetate in Owl's Hole

Compared to the Lucayans Caverns site (Figure 4.18), the minimum concentration was 0.40 μM with a maximum of 33.78 μM. Concentrations within the Owl's Hole site are decreasing with depth whereas in the Lucayan Caverns site the acetate concentrations are increasing with depth.

4:7 Radiotracer Results

4:7:1 ^{14}C Acetate Turnover

Turnover rates for radiolabeled ^{14}C acetate were positive for most depths within the vertical water column (Figure 4.45). Unlike the Lucayan acetate results, the 2-hour and 6-hour incubation rates of the Owl's Hole samples were very different in that the 6-hour incubations were substantially greater than the 2-hour incubations, although both the 2- and the 6-hour incubations for Owl's Hole were

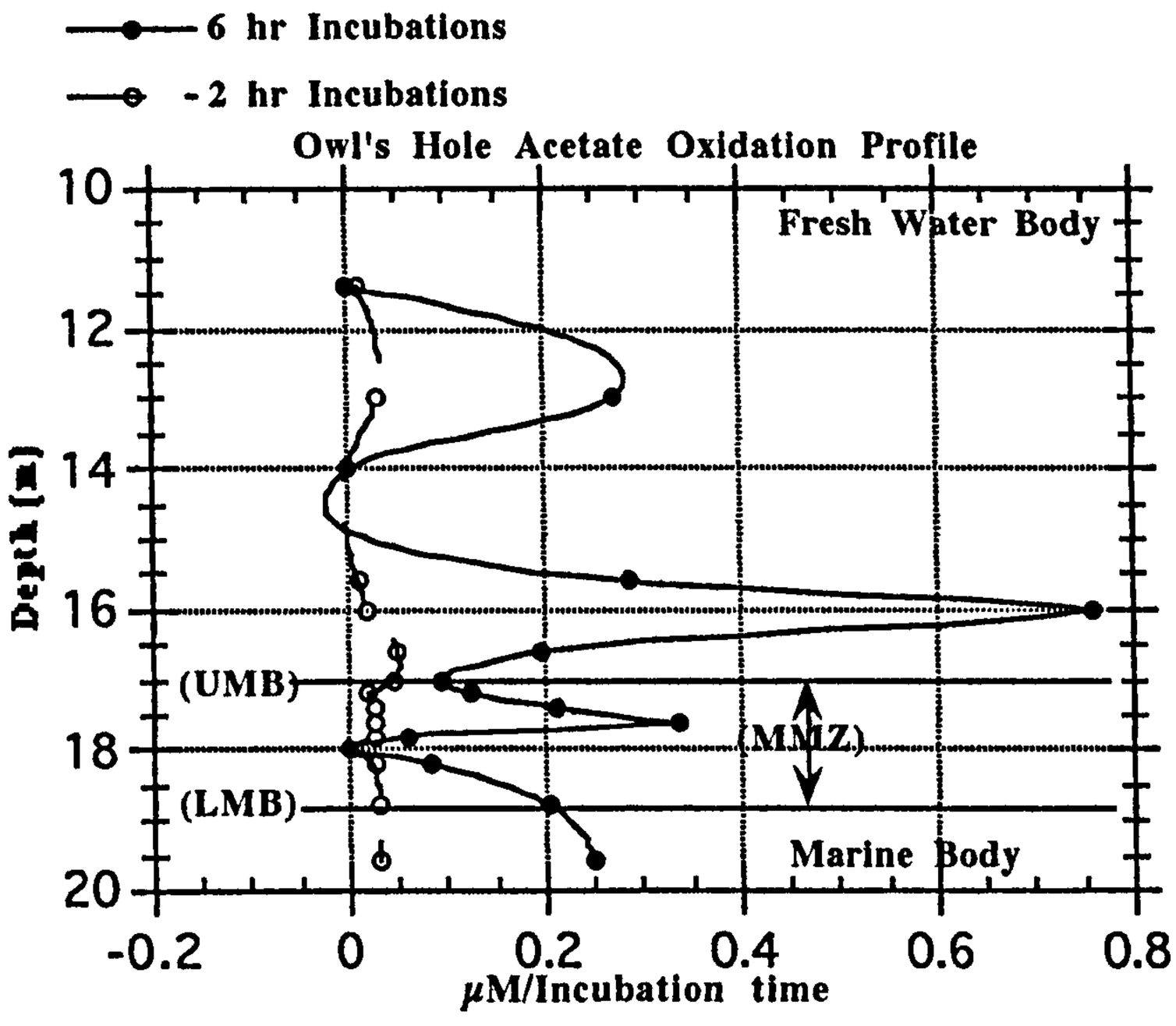


Figure 4.45: Potential acetate oxidation rates/hr. for the vertical water column in Owl's Hole

significantly less than the Lucayan results (see Figure 4.19). The turnover rate for the 2 hour incubation was from no activity to 0.1 μM. The turnover rate average for the 2 hour incubation was zero for the MMZ, and zero for the saline body.

The range for the 6-hour incubations went from zero activity to 0.8 μM/hr. The average for the 6-hour incubations were 0.3 μM/hr. for the fresh water body, 0.1μM/hr for the MMZ, and for the marine body, 0.3 μM/hr. Maximum activity for both, the 2-hour and the 6-hour incubations, occur at 13 m and around 16 m.

4:7:2 ^{14}C Bicarbonate Utilization

Positive evidence for bicarbonate incorporation occurred virtually throughout the water column (Figure 4.46) except for the most shallow 5-day incubation sample at 11.4 m which was zero. Two-day incubation rates were generally higher than the 5-day incubation rates, especially from 14 m to 16.6 m. Maximum activity occurs within the fresh water body and ends just prior to UMB depth.

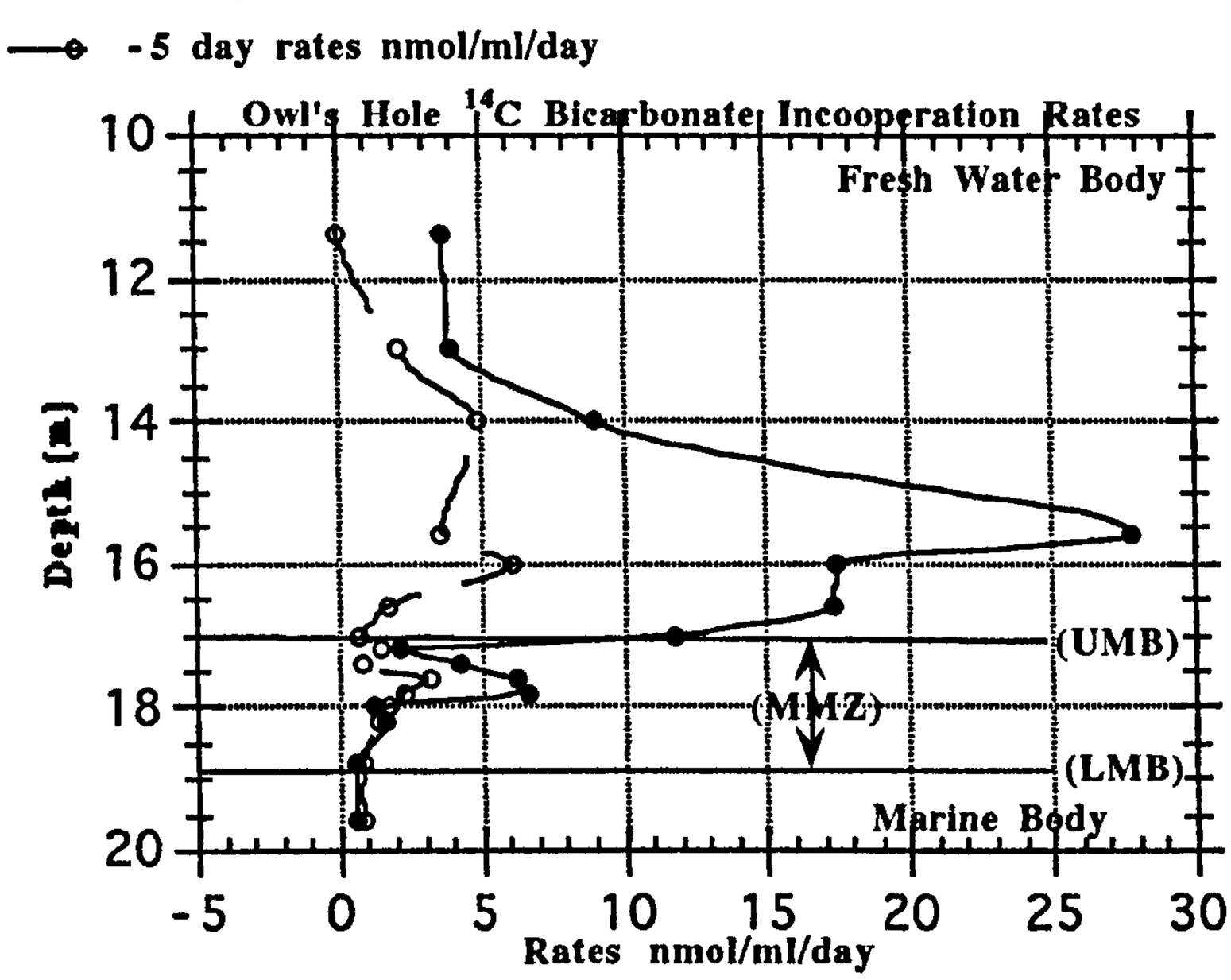


Figure 4.46: Potential ^{14}C bicarbonate uptake by bacteria within the vertical column in Owl's Hole

The range for the 2-day incubations was 0.51 nmol/ml/day as the minimum at 18.8 m and the maximum range was 27.8 nmol/ml/day at 15.5 m. The average for the 2- day incubations was 11.7 nmol/ml/day for the fresh water body, 3.4 nmol/ml/day for the MMZ, and 0.51 nmol/ml/day for the saline body.

The average incorporation rates for the 5-day incubations were 2.6 nmol/ml/day for the fresh water body, 1.7 nmol/ml/day for the MMZ, and 0.84 nmol/ml/day for the saline body.

4:7:3 Sulphate Reduction

The 2-day incubations, although varied, are higher than the 5-day incubation rates (Figure 4.47) as seen in Lucayan Cavernns. Maximum activity for the 2-day rate occurs at 16.2 m. In the Lucayan Caverns site a peak of activitys observed at the base of the MMZ.

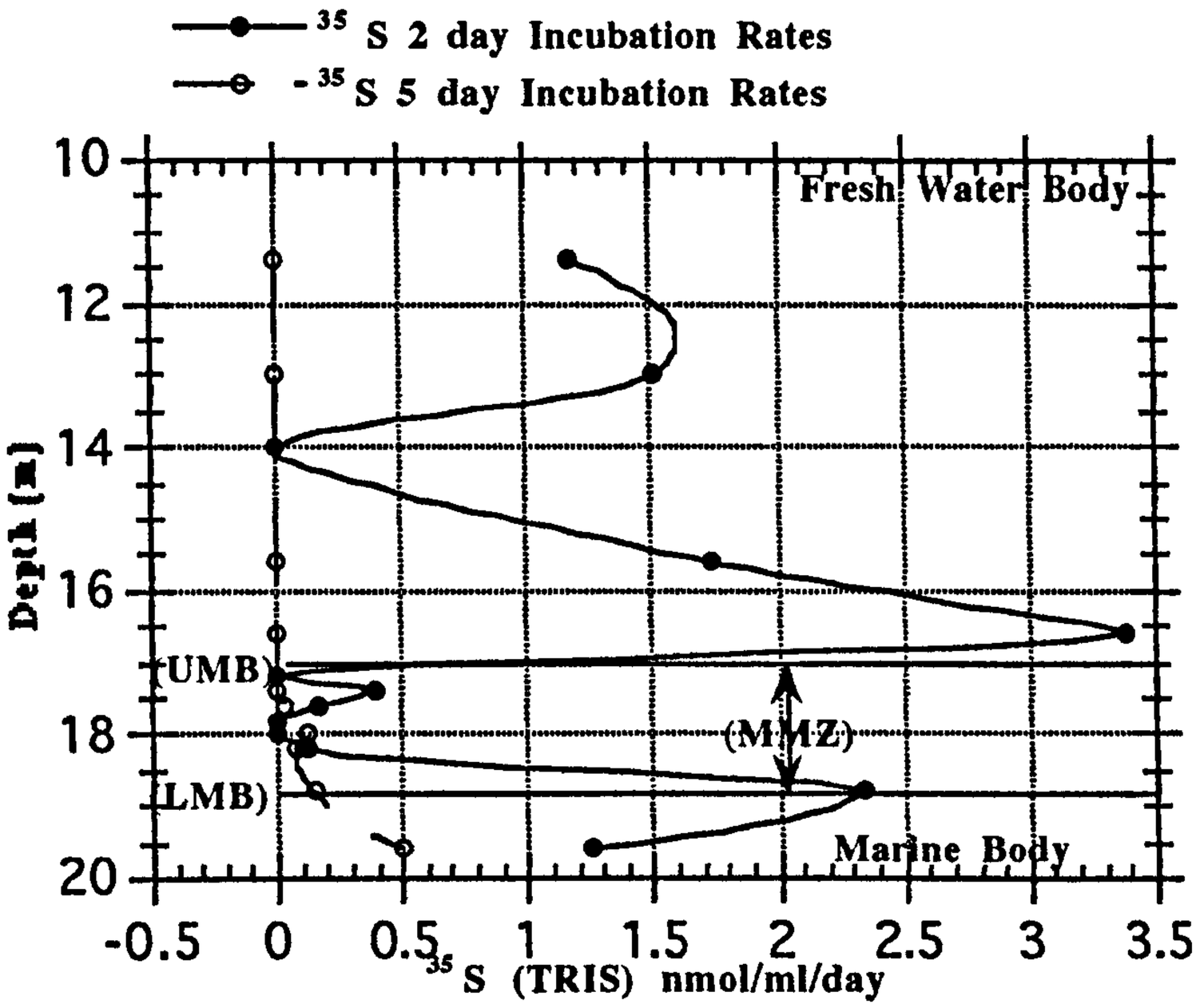


Figure 4.47: Potential ^{35}S (TRIS) reduction rates within the vertical water column in Owl's Hole

Although the peak activity for the 2-day incubation rate at the Owl's Hole site is above the mixing one, taking into account a 1 m tidal fluctuation would put the peak at the Owl's Hole site near the LMB within the MMZ; similar to the Lucayan Caverns site. This is speculation at this time but will be discussed further in chapter 6.

A second peak of activity for the 2-day incubation occurred at 18.8 m at the LMB. However, following this peak, the rates drop where the 5-day incubation rates demonstrate comparatively maximum activity. All previous 5-day incubation rates indicated no or little activity. When plotted against oxygen concentrations (Figure 4.48) the 2-day rate activity occurred seemingly irrespective of O_2 levels, but the 5-day rates became comparatively significant when O_2 levels dropped below 1 mg/l. An important aspect is that reduction rates are being plotted against O_2 levels, measured within 30 minutes to 1 hour of recovery from the cave. Samples incubated for sulphate reduction

measurements would continue to consume oxygen within the sealed vials and hence oxygen concentration would be lower than those *measured in situ*.

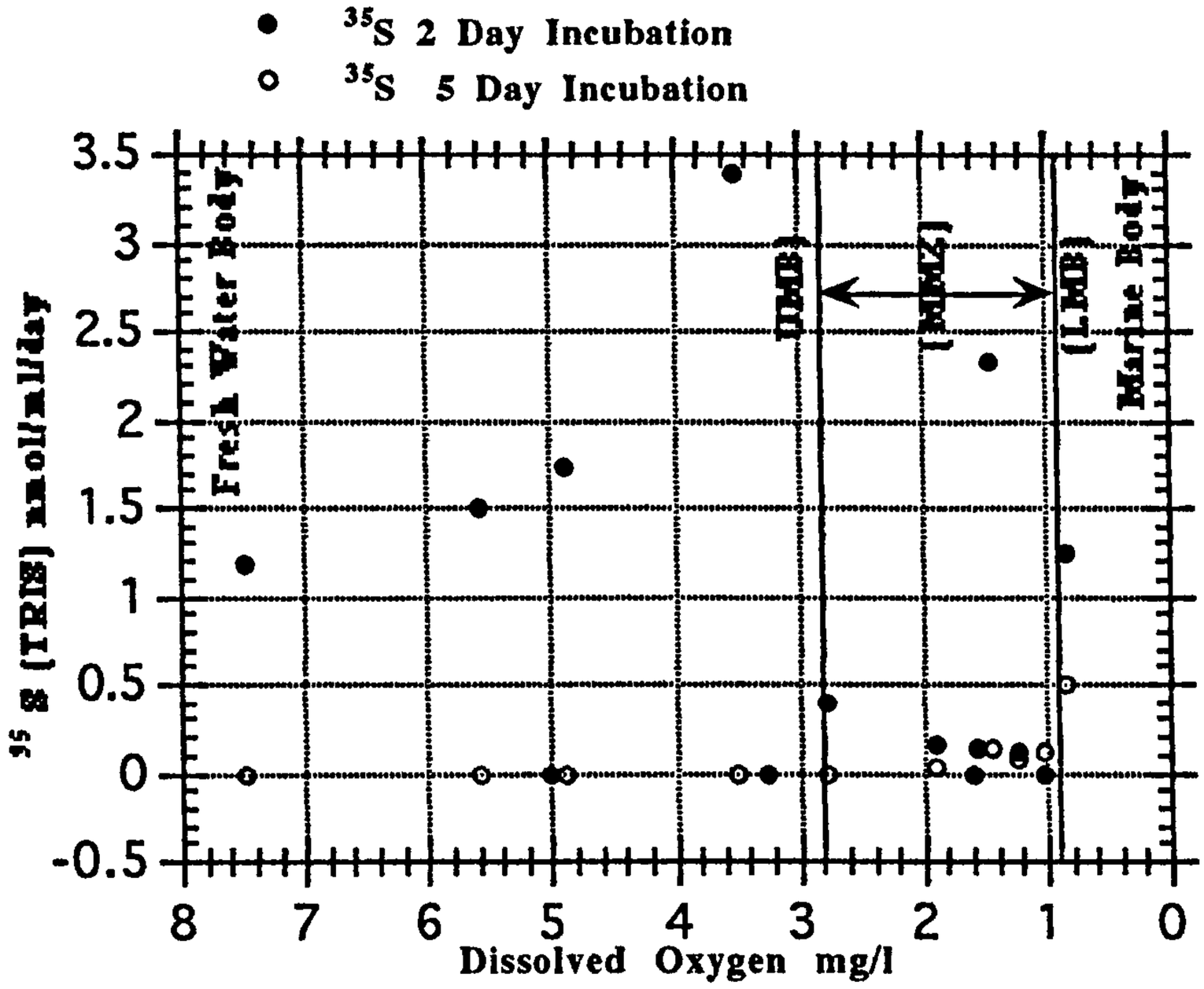


Figure 4.48: Owl's Hole sulphate reduction results plotted against oxygen mg/l

4:8 Bacterial Numbers

4:8:1 Total Bacterial Cell Numbers

Bacteria were found in all water samples throughout the site (Figure 4.49). Distributions throughout the water column were varied but more so within the MMZ. The numbers ranged from the minimum of 10,524 cells/ml at 16 m to a maximum count of 216,321 cells/ml at 14 m. The average cell numbers for the fresh water body were 92,460 cells/ml the MMZ, 39,972 cells/ml and in the marine body 91,285 cells/ml. Based on these counts the majority of the bacterial population were found within the fresh water body and the marine sections of the water column, not the MMZ.

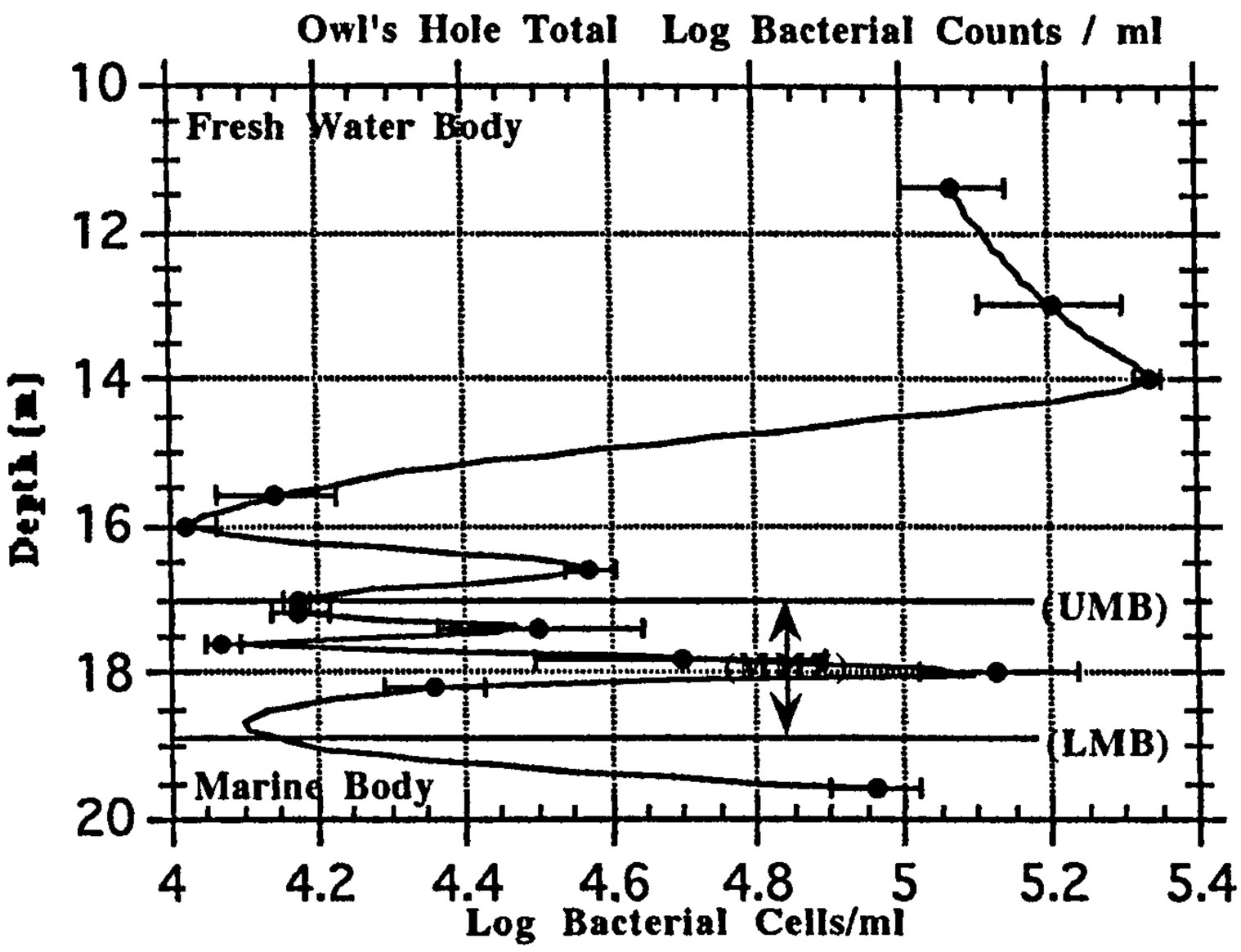


Figure 4.49: **Log bacterial counts / ml for Owl's Hole water sample site. Error bars represent the range of measurements.**

The UMB and LMB boundary does not seem to influence bacterial numbers, in fact, they are the sites of low bacterial numbers.

4:8:2 Numbers of Dividing Cells

Dividing cell numbers throughout the water were variable except from 18 m and deeper (Figure 4.50). The most varied counts were within the mixing zone. The percentage value for dividing cells was 3.87% at 13 m and 31.07% in the marine body at 19.6 m. The average concentrations for the fresh water body were 9.25%, 14.45% for the MMZ, and 31.07% for the marine section of the water column.

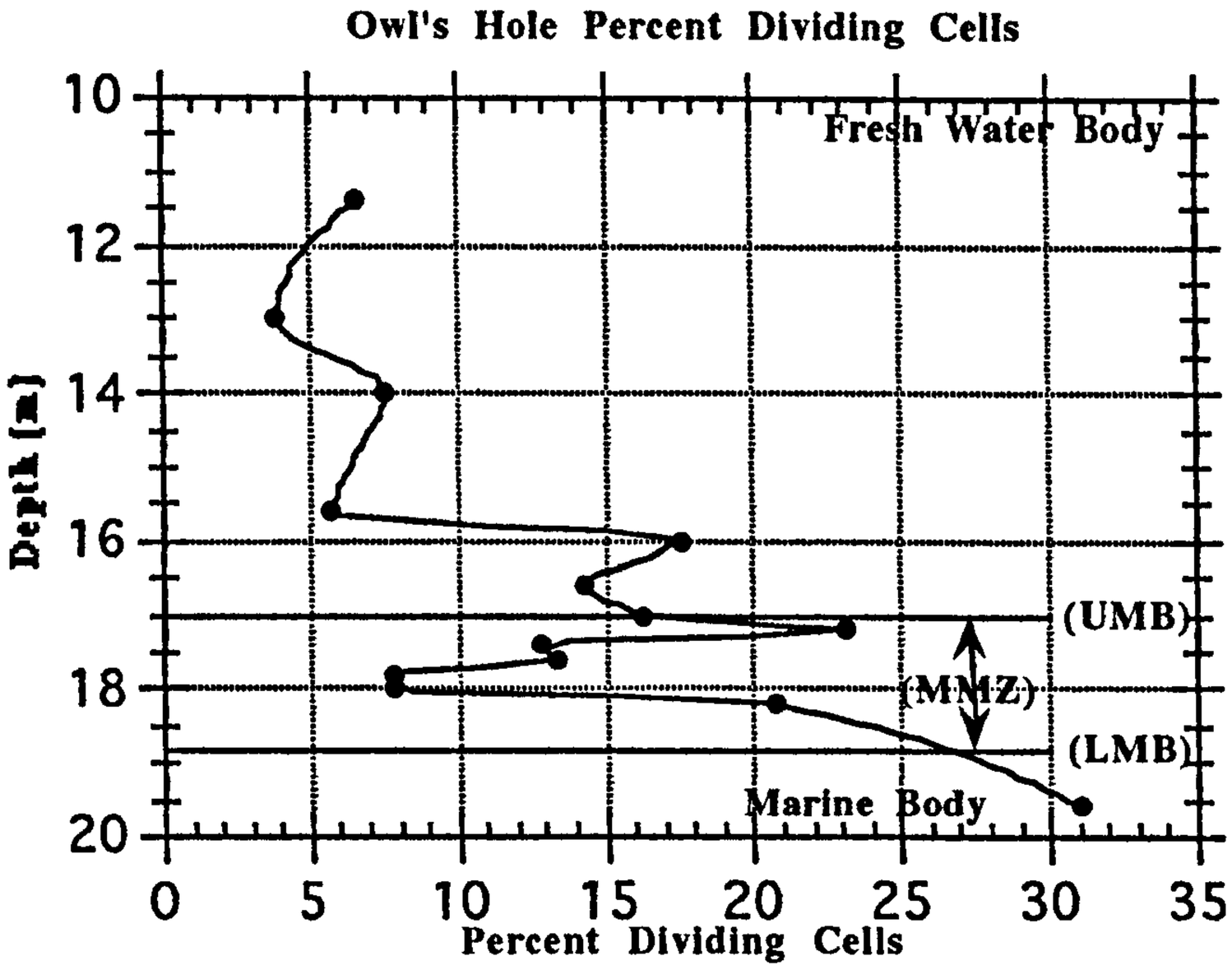


Figure 4.50: Percent dividing cells in the Owl's Hole sample site

Percentage dividing cells of the total population demonstrated that the percent dividing cell numbers are increasing with depth and with maximum activity within the marine zone.

4:8:3 Bacteria Counts on Particles

Bacteria on particle counts were variable from 14 m to 18.8 m. (Figure 4.51). From 11.4 m to 13 m the counts decreased gradually. From 14 m the counts became very variable, especially within the MMZ. The elevated counts within the MMZ do not appear to be responding to the major density interface of the UMB and the LMB, yet the peak counts occur directly beneath the UMB and just above the LMB.

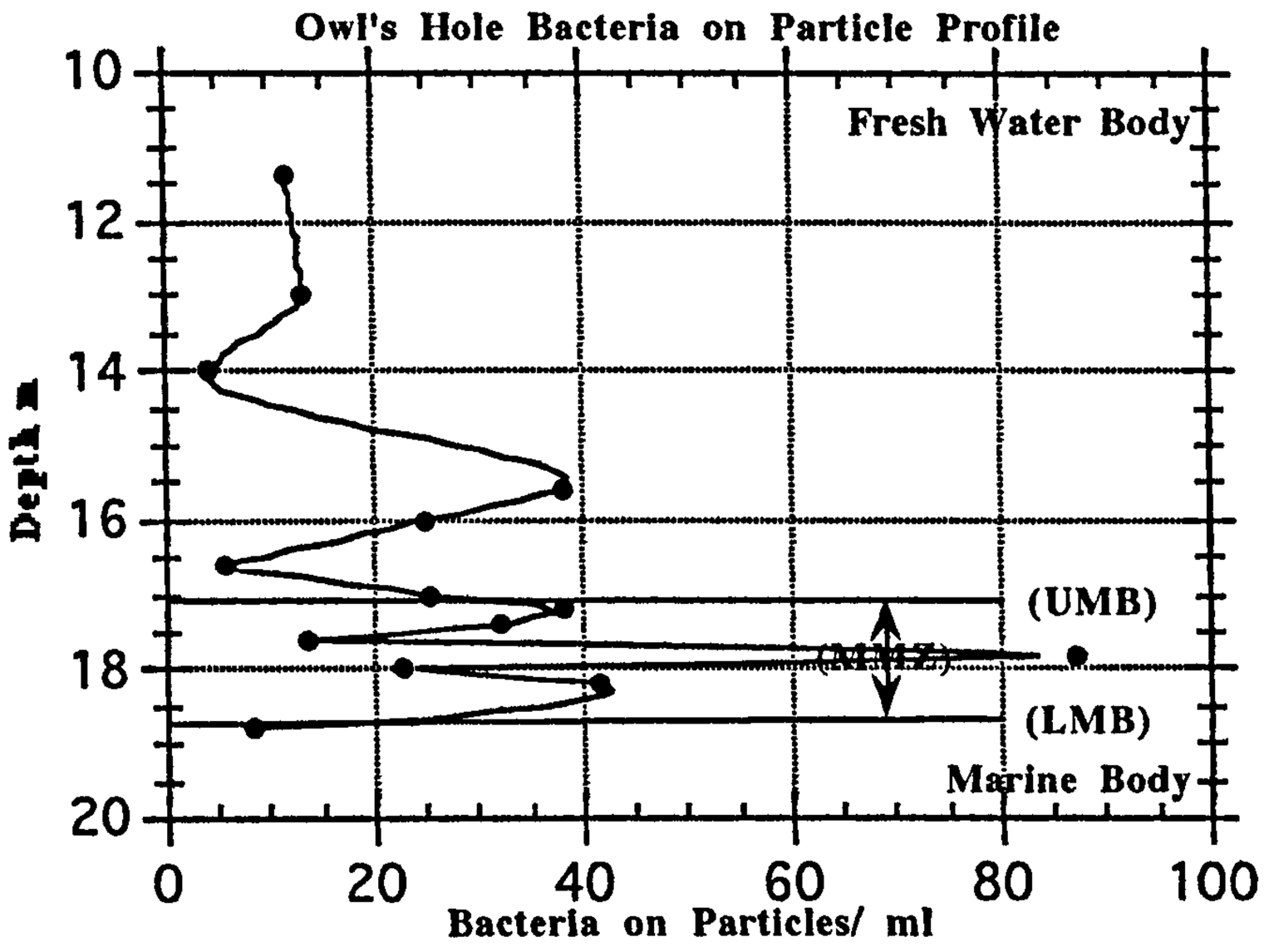


Figure 4.51: Owl's Hole bacteria on particle profile throughout the vertical sample site.

The maximum cell numbers occurred within the middle of the MMZ. The on particle cell numbers ranged from 4.33 at 14 m to 87.33 counts at 17.8 m. The fresh water body average was 18 cells/ml, and the MMZ was 32 cells/ml.

4:8:4 Bacteria Counts off Particles

Bacteria cells off particle decreased with depth (Figure 4.52). The counts in the samples from 11.4 m to 16 m decreased gradually, however, the counts from 16 m and deeper varied dramatically till the last counted sample in the marine zone. Bacterial cell numbers ranged from a minimum of 280 cells at 17.6 m to a maximum cell number of 439 at 13 m.

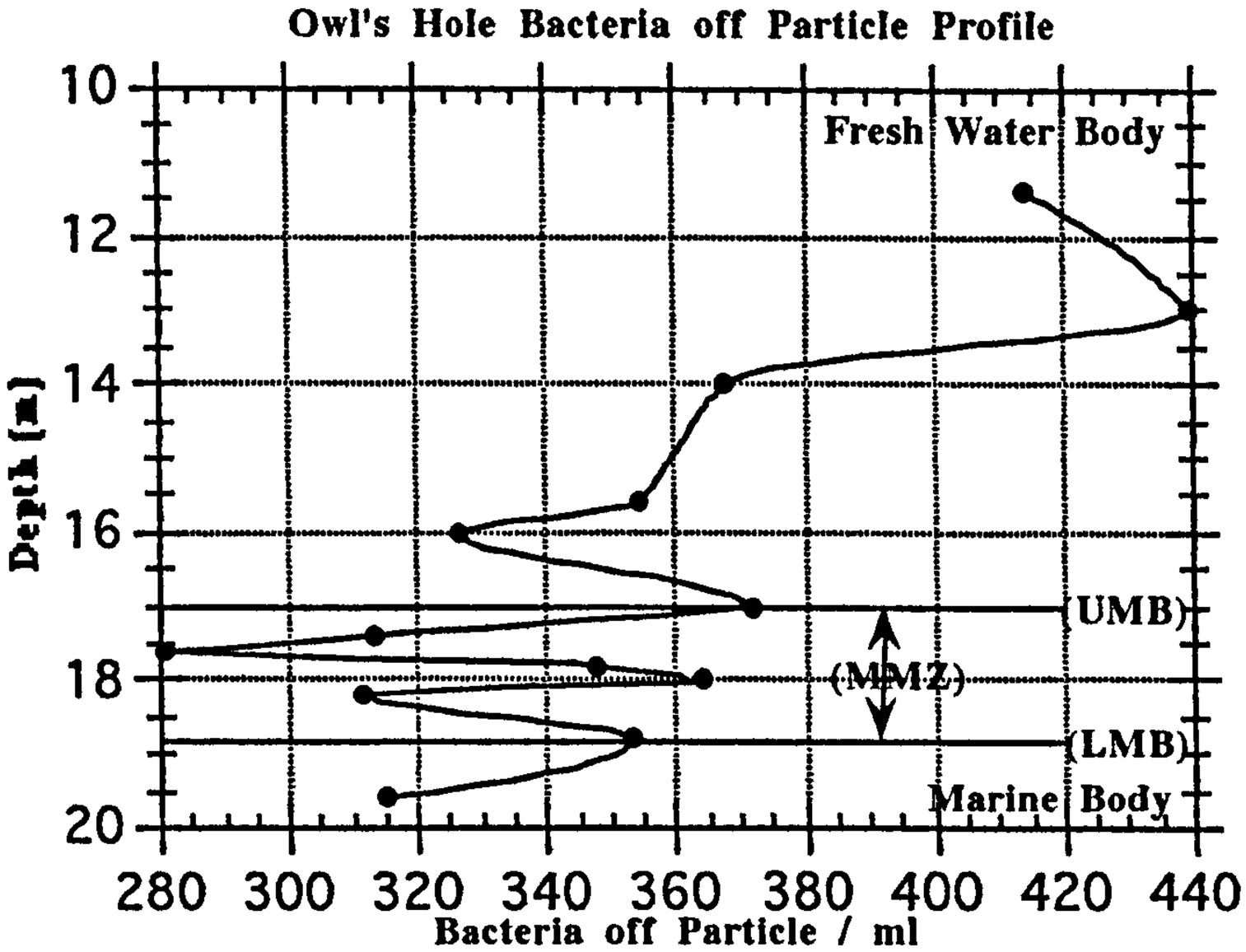


Figure 4.52: Bacteria off particle / ml profile for the Owl's Hole sample site

The mean of the counts within the fresh water body are 380 cells/ml, 334 counts/ml, in the MMZ, and 315.33 counts/ml in the marine body. The UMB and the LMB have elevated counts which may or may not be a result of the major density interface at these depths.

5
Field Season 2 (February 1996) Samples from Lucayan Caverns Grand Bahama

Introduction

The objectives for collecting samples again at the Lucayan Cavern site (appendix 1) was to try and answer the following questions: were the bacteria found within the Lucayan sample site on previous collection trips viable, and could we measure this activity using radio-labeled [methyl $^{-3}$ H] thymidine? Geochemical parameters such as temperature, salinity etc., were measured again to (1) create a framework of background data for thymidine results and (2) identify any geochemical changes which may have occurred since 1994, and in some instances since 1990, when samples were first collected from these sites. Samples from Stargate, a cave not previously sampled for this project, had all the water analyses done, i.e., geochemistry, radiotracers, etc., because no prior data existed from this project. Stargate, a fracture cave system, (refer to section 2:2:3) located in a town named "The Bluff" on South Andros Island in the Bahamas, was of interest to this project to see if a fracture system would prove to be different in respect to both abiotic and biotic processes, if compared to those processes understood to occur in lens based cave systems. The 1998 hydrolab data is again used to compare results generated over the 4-year-period and in some instances, 12-year period.

5:1 1996 Geochemistry of the Lucayan Caverns

5:1:1 Salinity

In the 1996 data group, 6 additional sample depths were examined, in addition to those in the 1994 study. Although the two data sets overall appear almost identical (Figure 5.1), the 6 additional samples helped to fill in gaps in the 1994 data set. Both the UMB and the LMB were as in 1994 at 14 m and 16 m respectively. These demonstrate that, at least over short periods of time, the UMB and the LMB are stable features as reflected in geochemical parameters such as temperature, alkalinity, dissolved oxygen and salinity.

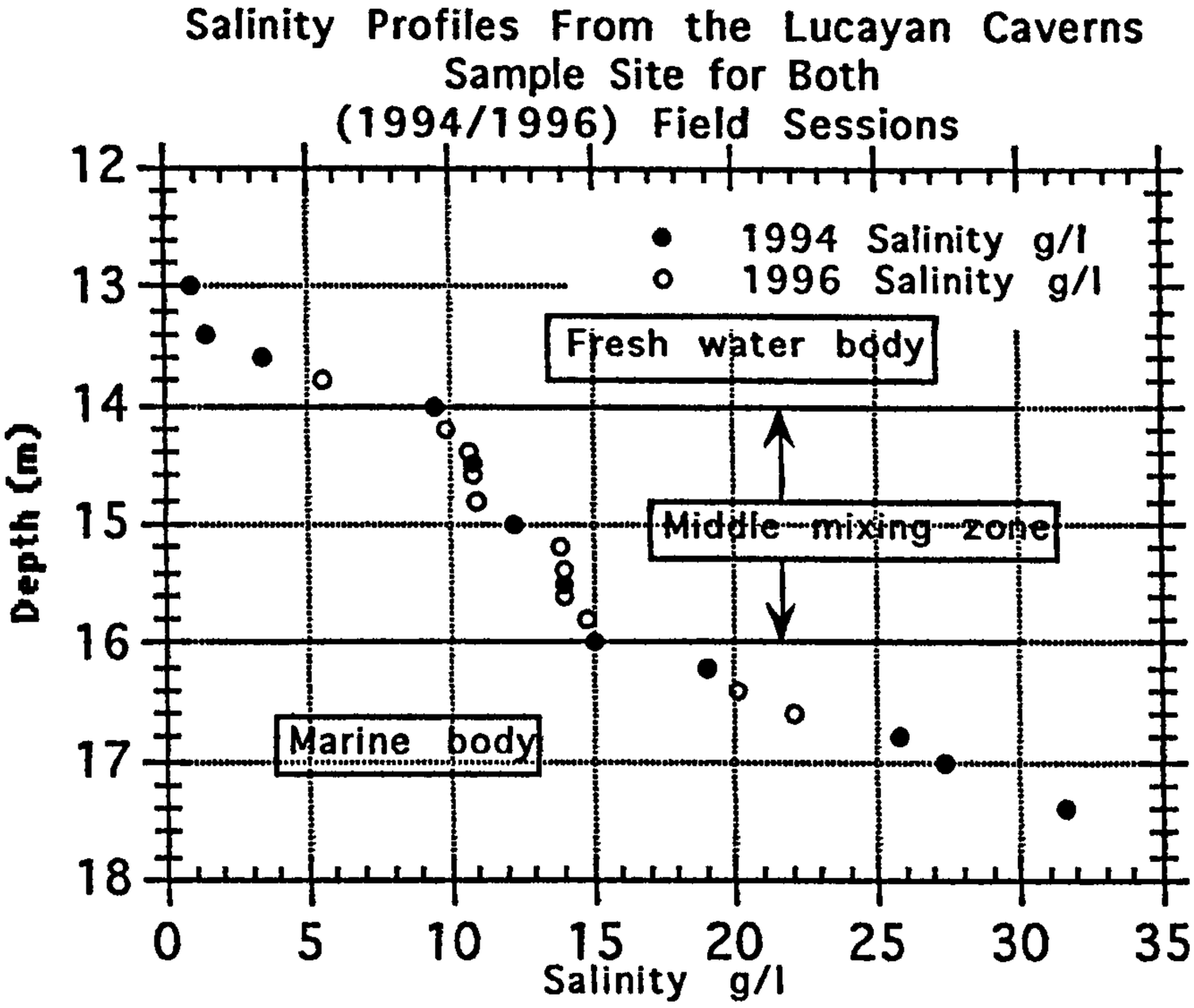


Figure 5.1: (1996) Salinity profile from the Lucayan Caverns.

Comparing the 1994/96 data with the 1998 hydrolab results (refer to figure 4.16(A)), the results are again very similar. Over a period of 4 years, location of the major salinity-boundaries appear not to change within the Wedding Hall sampling site.

5:1:2 Temperature

The temperature profile for Lucayan Caverns in 1996 (Figure 5.2) was almost identical to the 1994 results. Temperatures in depth from 13.4 m to 16 m are only slightly cooler. Sampling techniques on both occasions were the same, and so was the air temperature, which was in the mid to upper 20°C. In 1996, however, samples were obtained over a period of 2 hours, whereas in 1994, samples were collected over several days and hence potentially subject to small-scale daily temperature fluctuations.

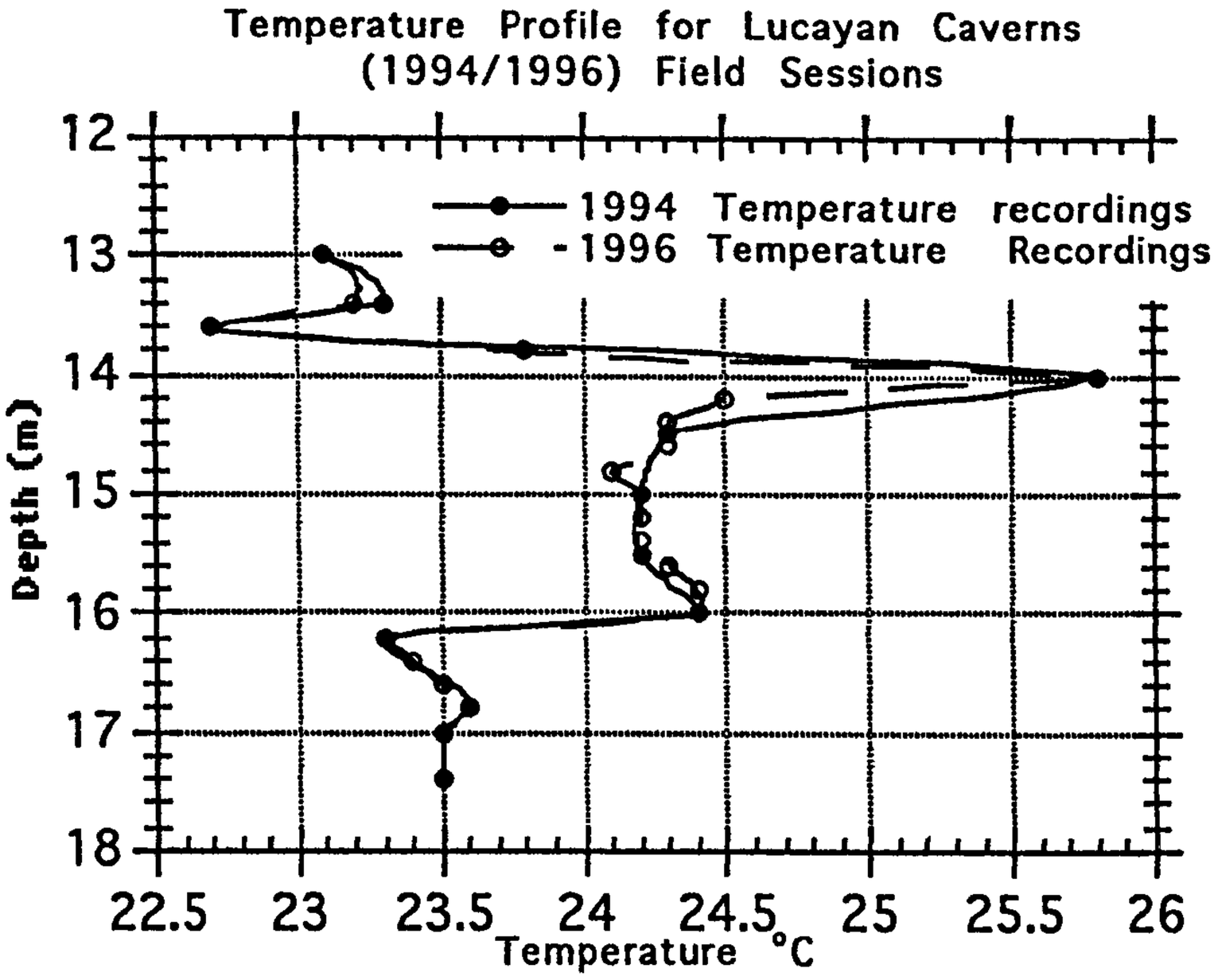


Figure 5.2: (1996 and 1994) temperature profile plotted with depth.

Comparing the 1996 temperature results with the 98 hydrolab profiles, (refer to figure 4.8) little has changed. The same general trend exists where there is a rapid increase in temperature up to the UMZ, stable temperature recordings within the MMZ, and once into the marine section, temperature decreases slowly.

5:1:3 pH

The overall pH measurements collected on the 1996 field trip (Figure 5.3) are more alkaline than the ones collected in 1994. However, the 1998 hydrolab data were the most alkaline so far measured within the Wedding Hall Room. Over a period of 4 years, for example, samples collected at 14 m, in 1994 pH results were pH 7.20, 1996, pH 7.48 and in 1998, pH 7.82. Again consecutive results for 16 m was pH 7.19 in 1996, pH 7.34 and in 1998, the pH measurement was 7.84.

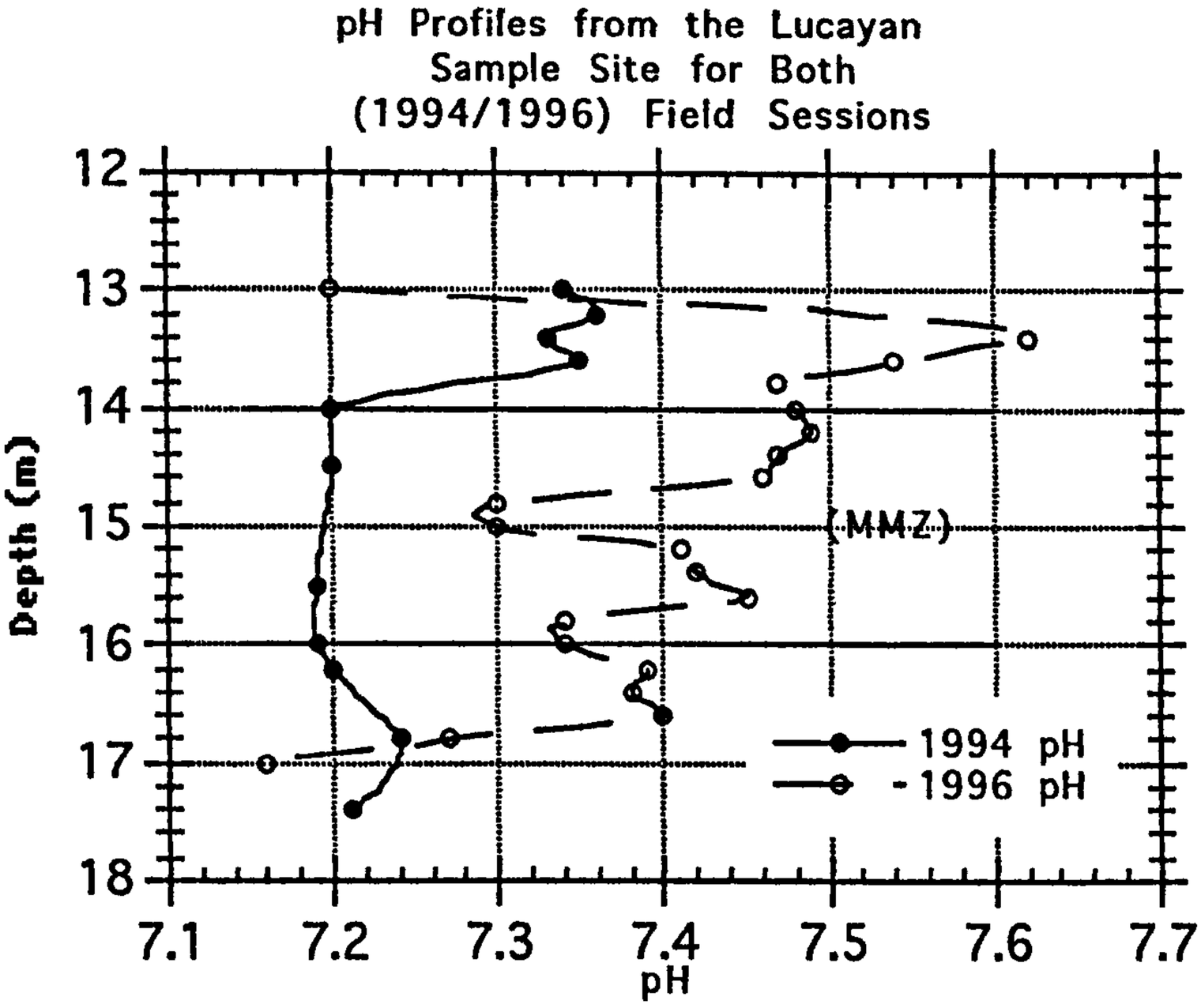


Figure 5.3: pH profile for the 1996 water samples from the Lucayan Caverns

The results show that over a 4-year period the entire water column has become more alkaline, with the fresh water lens being the most alkaline, but with depth the water column becomes more acidic with marked changes across the UMB and the LMB.

5:1:4 Alkalinity

Alkalinity depth variations for both the 1994 and the 1996 field collections provided similar test results, although the 1996 values (Figure 5.4) are higher than the 1994 values by 20 $mmol^{-1}$ $CaCO_3$. The alkalinity for the 1996 measurements ranged from 250.1 mmol-1 $CaCO_3$ at the most shallow sample site to 165.9 $mmol^{-1}$ $CaCO_3$ from the deepest marine sample site. In comparison, the Lucayan 1994 samples, the most shallow sample which was also from 13 m, had a measurement of 231.1 $mmol^{-1}$ $CaCO_3$. The highest alkalinity reading from 1994 however, came from the 13.2 m sample site. The 1994 lowest alkalinity was from 17.4 m with a measurement of 163.1 $mmol^{-1}$ $CaCO_3$. This sample is 0.4 m deeper than the deepest sample collected in 1996.

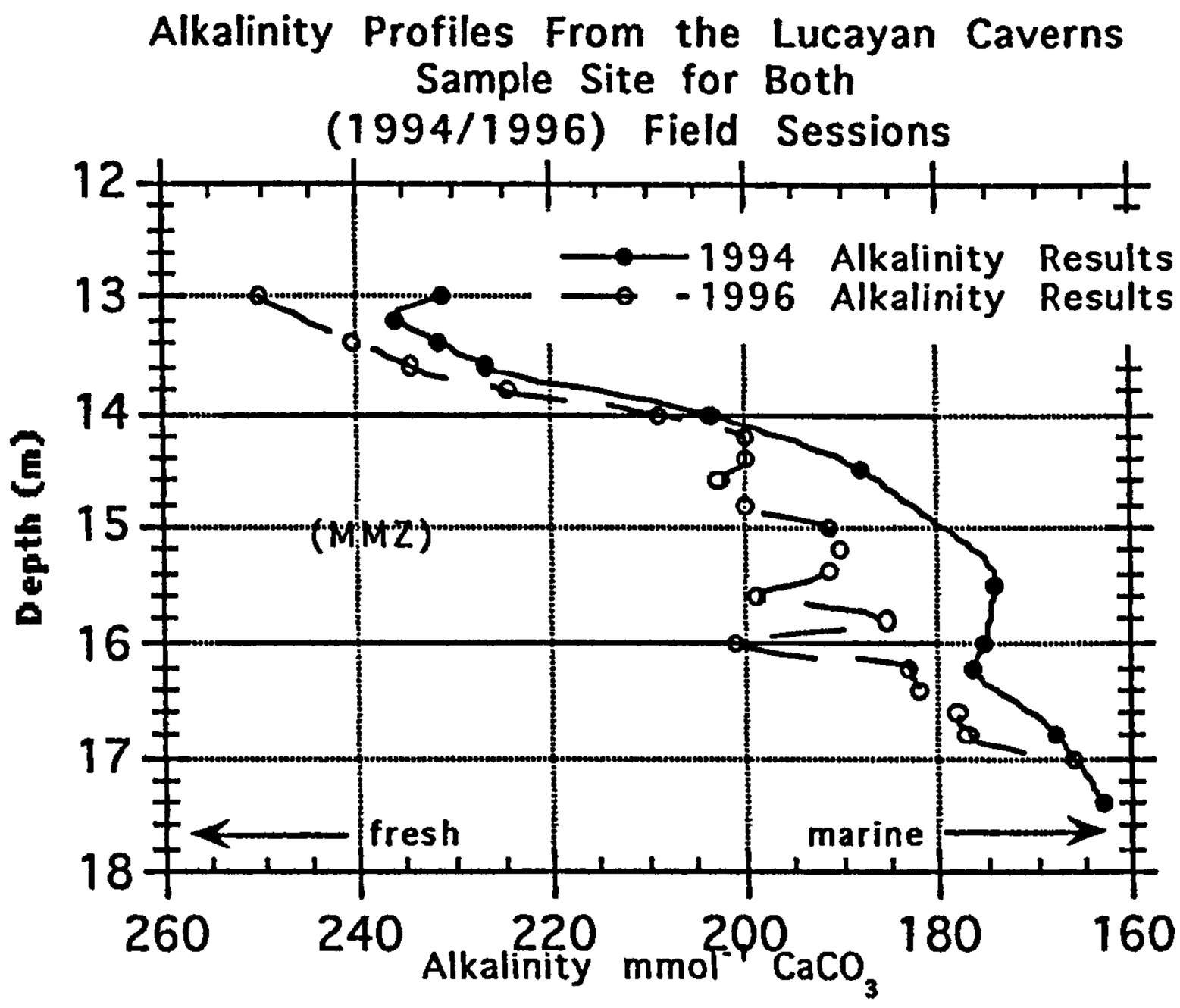


Figure 5.4: Alkalinity profile for the 1996 water samples from the Lucayan Caverns

The greatest difference between the two data sets is within the MMZ. The additional samples obtained in 1996 demonstrate greater variability in this zone, if compared to 1994. If only the same sample depths in 1994 and 1996 within the MMZ are considered then the two profiles are broadly similar despite the higher alkalinity in 1996. This demonstrates the need for good depth resolution and comparing general trends rather than viewing the visual trends when there are only a few data points.

5:1:5 Dissolved Oxygen

Again, the 1996 DO results (Figure 5.5) almost duplicate the 1994 results except that overall, the 1996 DO levels are slightly higher than the 1994 results. In the 1996 data, at the lower portion of the MMZ, between 15.2 m and 15.4 m, the DO concentrations drop 20 cm by 1 mg/l in contrast to the 1994 data which appeared relatively constant over this depth interval.

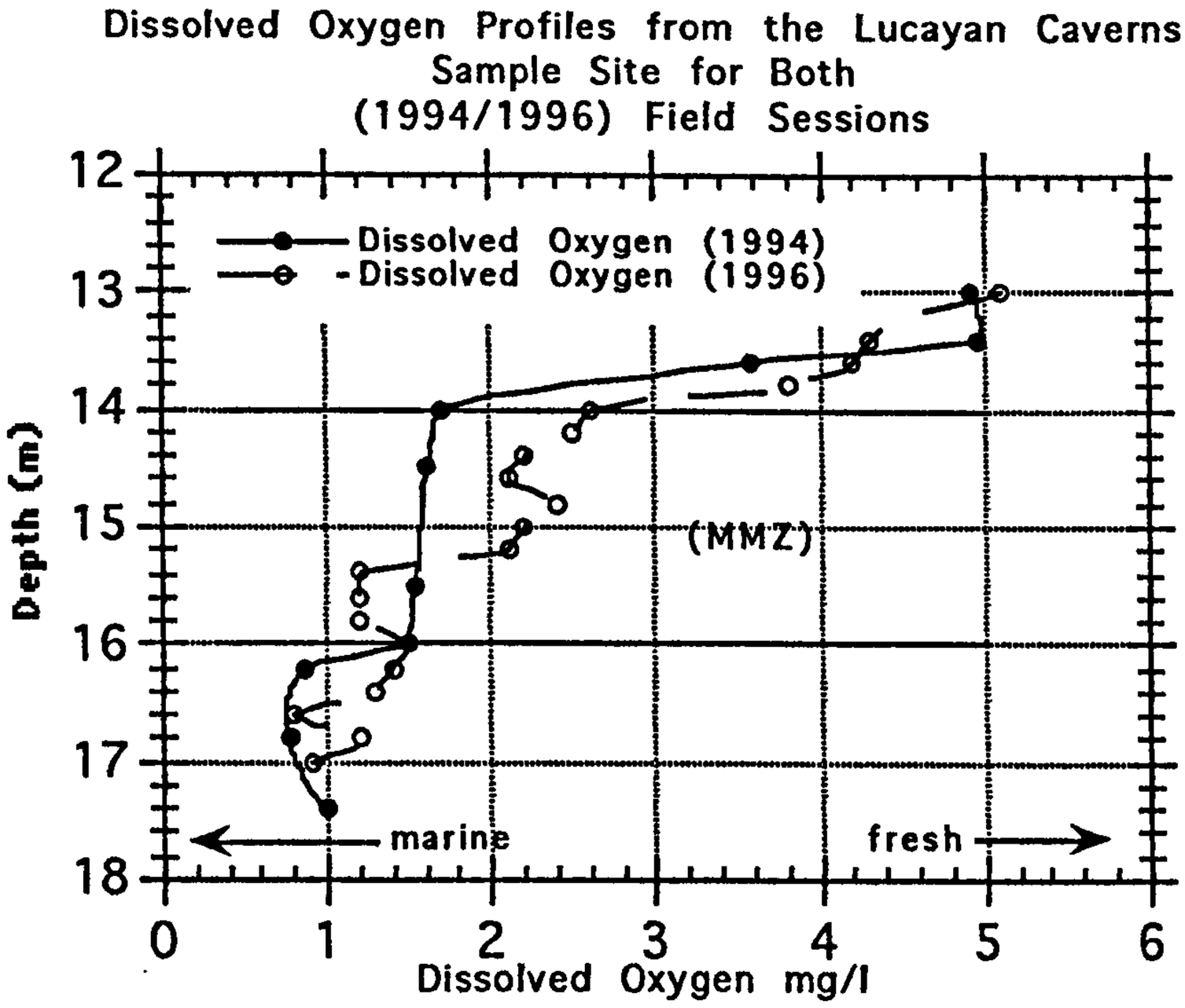


Figure 5.5: Dissolved oxygen profile for the 1996 water samples from the Lucayan Caverns

Only one data point from 1996 matched the 1994 point numerically and that was at 16 m where both samples had DO levels of 1.45 mg/l. The hydrolab DO results were significantly higher than those obtained during both previous field sessions. This has interesting implications in reference to field sampling methods. A great deal of care was taken to make sure that water samples did not take on oxygen once removed from the cave. The hydrolab results are taken *in situ* and the DO measurements are significantly higher than those results measured outside of the cave environment. Over the last 4 years the DO levels have either increased or DO was lost from the samples which is highly unlikely because atmospheric PO_2 is significantly higher outside of the water sample bags than inside (refer to figure 3.2).

5:1:6 Dissolved Organic Carbon

The dissolved organic carbon results between the two field sessions appear radically different (Figure 5.6). The 1994 data fluctuates throughout the water column whereas the 1996 values gradually decrease with depth with two small increases consecutively at 14.8 m and 16 m, the LMB. At 14.8 m there is a 4 mg/l increase from 14.6 m to 14.8 m and an increase of 7 mg/l from 15.8 m to 16 m. Again, the 7 mg/l increase is in association with the LMB.

It is difficult, if not impossible, to compare the 94/96 data sets. Possible explanations for the 1994 data being so different will be given in the discussion in Chapter 6.

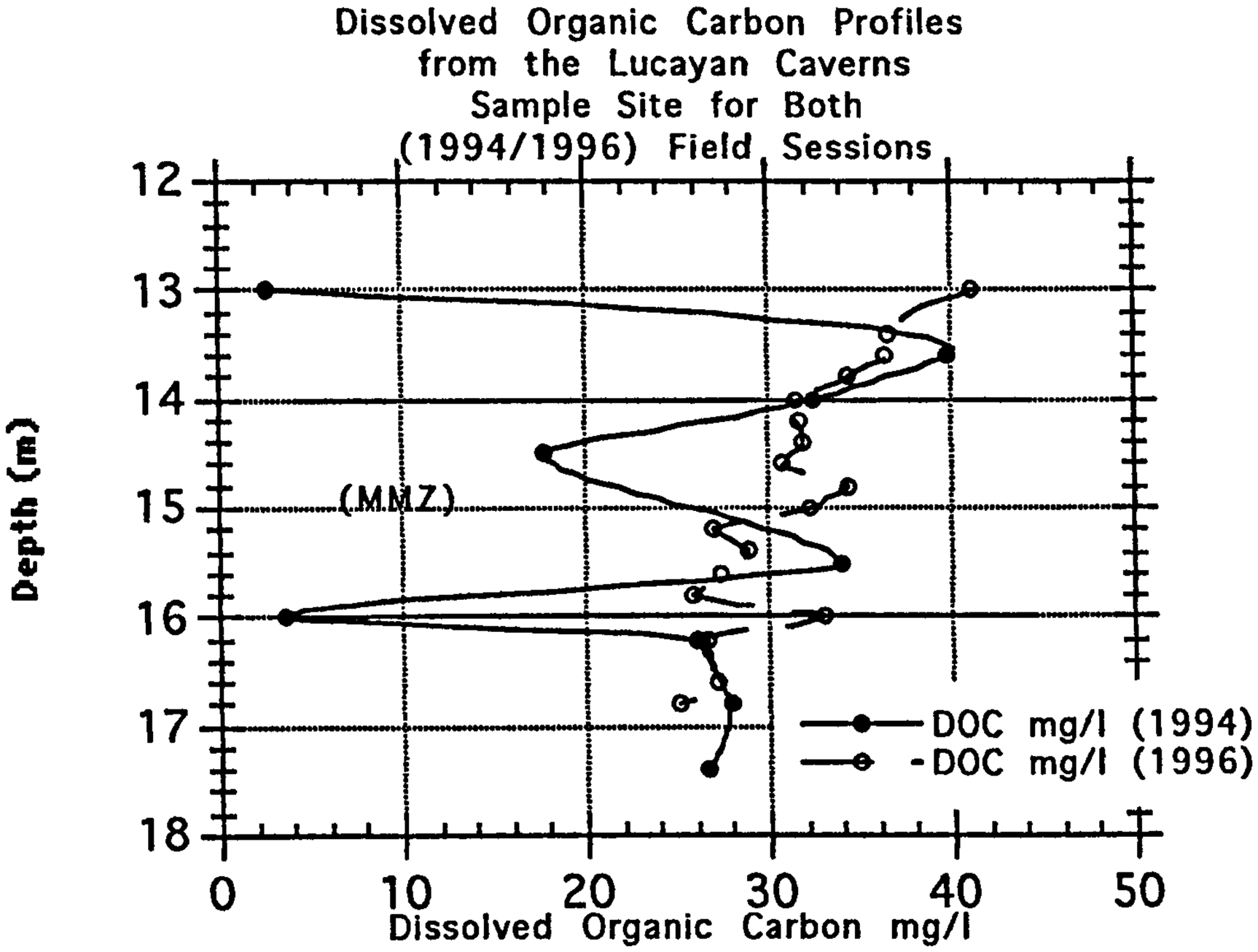


Figure 5.6: Dissolved organic carbon measurements for the 1996 Lucayan Caverns' water samples

5:1:7 Particulate Organic Matter (POM)

The amount of particulate organic matter (POM) recovered from the 1996 samples (Figure 5.7), ranged from 0 mg/l at 13 m to 0.031 mg/l at 16 m at the LMB. The data from 16 m tie in with the results from the 1996 DOC (Figure 5.6) where at 16 m a peak in POM occurred also.

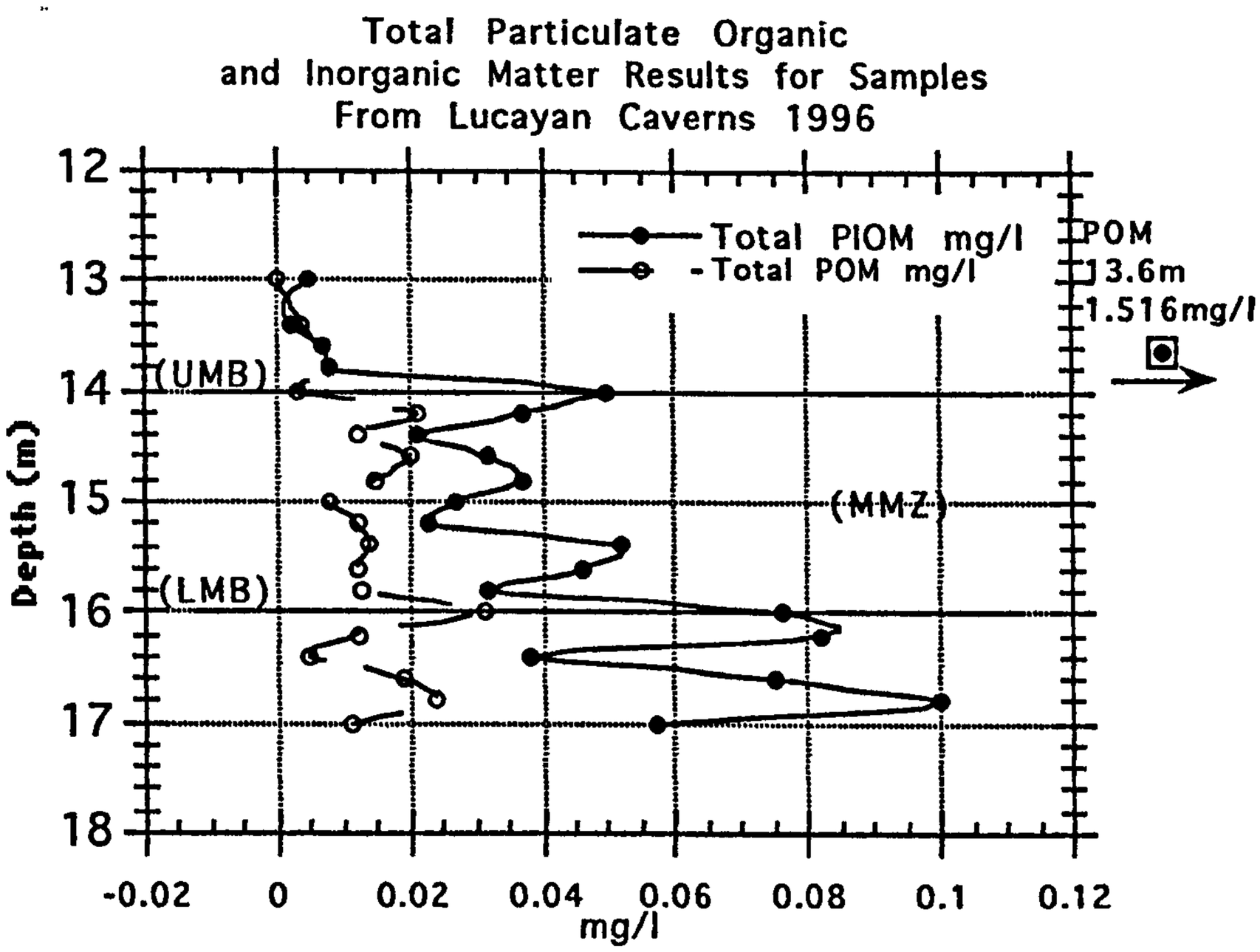


Figure 5.7: Particulate organic matter (POM) from the 1996 water samples from the Lucayan Caverns

Throughout the water column the amounts of POM are small, but the small amounts were reasonable considering the clarity of the waters within these caves. The results for particulate inorganic matter (PIOM) are higher than the POM results. As expected, as salinity increases, PIOM value increases. Where the salinity steps occur at the UMB and the LMB, PIOM values increase sharply.

At 13.6 m, a value for POM of 1.516 mg/l is marked outside of the graph in a box. It is unclear if this is a real value or erroneous. As a result of the values being so small overall, to plot the point at 13.6 m would have given a straight line graph and the small values of the rest of the water column could not be examined. For this reason, the point was labeled separately.

5:2 Radiotracer Results

5:2:1 Bacterial Growth Rates, Incorporation of Tritiated Thymidine Into (DNA)

All samples collected from the Lucayan sample site were positive for thymidine activity (Figure 5.8). Rates of thymidine incorporation into DNA ranged from a minimum of 7,230 cells/ml/day at 13.6 m, to a maximum, 379,012 cells/ml/day at 16.2 m. Peak activity appears to be at 16 m, just below the LMB. Within the MMZ, activity remained elevated, and within the fresh water body and the marine section of the water column there appears to be more localized activity.

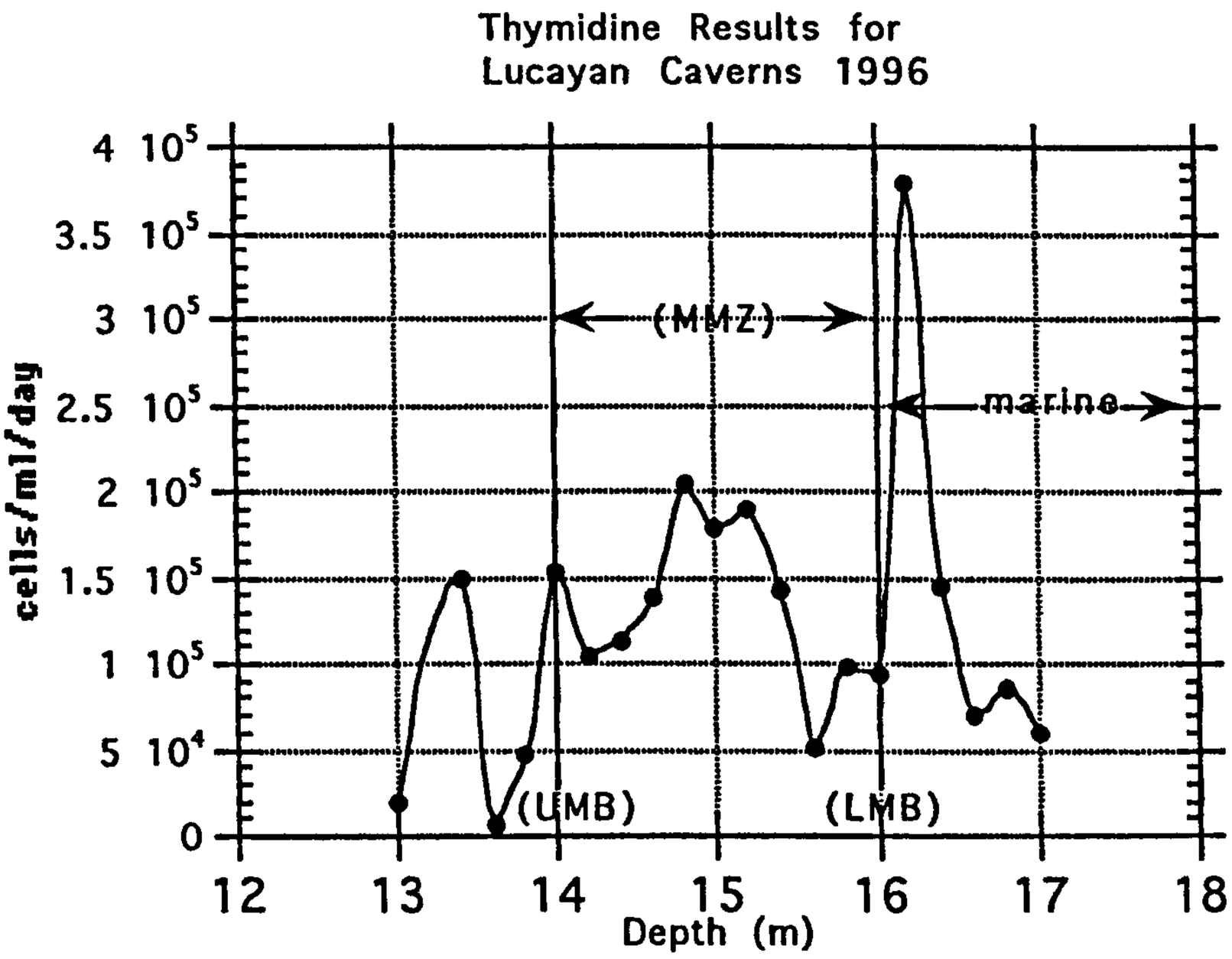


Figure 5.8: Thymidine incorporation rates for water samples collected in the Lucayan Caverns, 1996.

Contrasting growth with percent dividing cells demonstrates some comparability between profiles within the MMZ. Overall, however, the distribution of dividing cell data does not correspond to that for bacterial growth. The total bacterial population had a similar distribution to bacterial growth with peaks at 13.4 m and a smaller peak at 16.4 m.

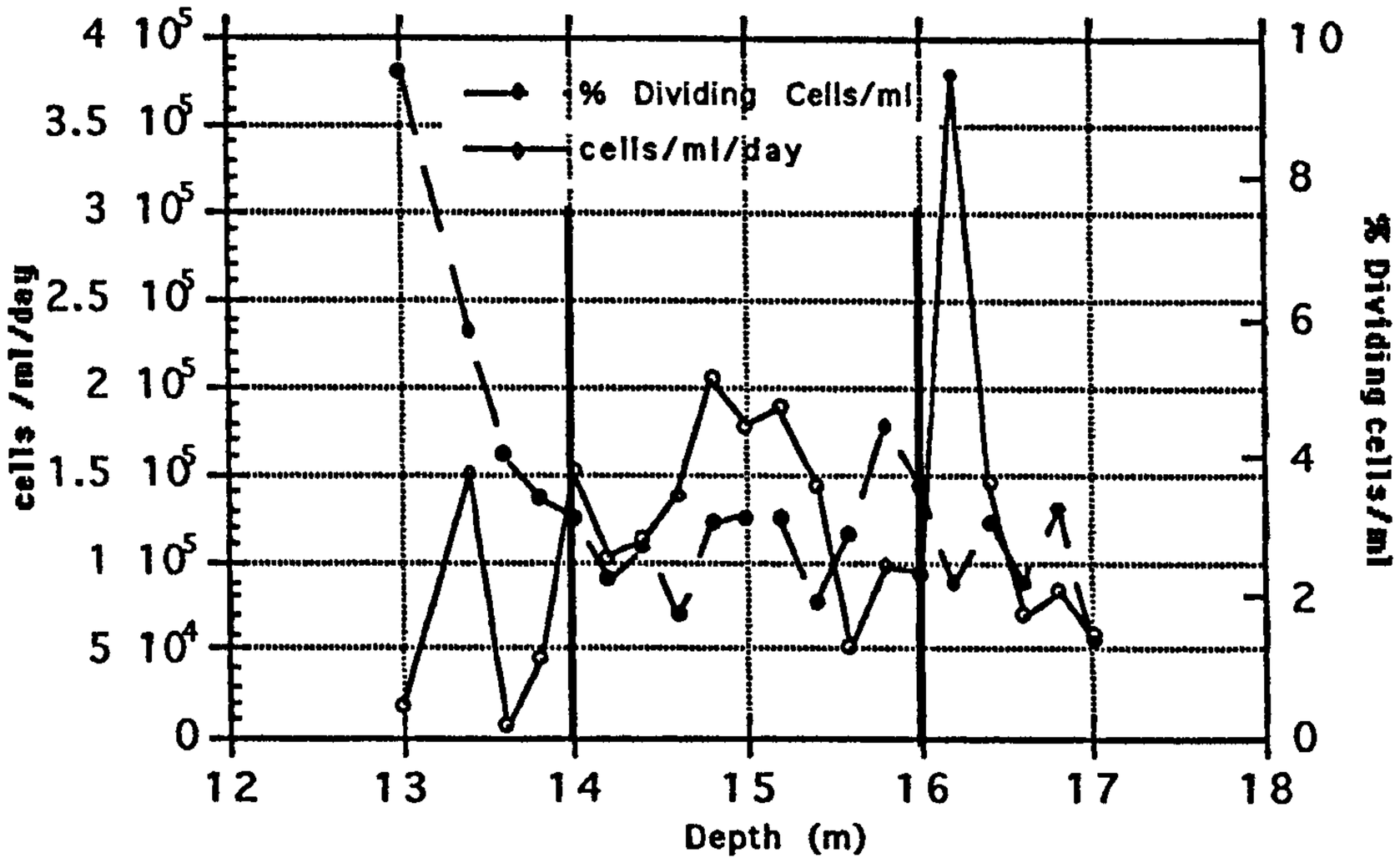


Figure 5.9: Thymidine incorporation rates and percent dividing cells for the 1996 Lucayan Caverns samples

5:3 Bacterial Counts

5:3:1 Total Bacterial Population

The total bacterial population (Figure 5.10) was at a maximum within the fresh water zone and were the highest within the vertical water column. A significant decrease in bacterial numbers occurred just above the UMB; here the counts were the lowest (27,114 bacteria/ml).

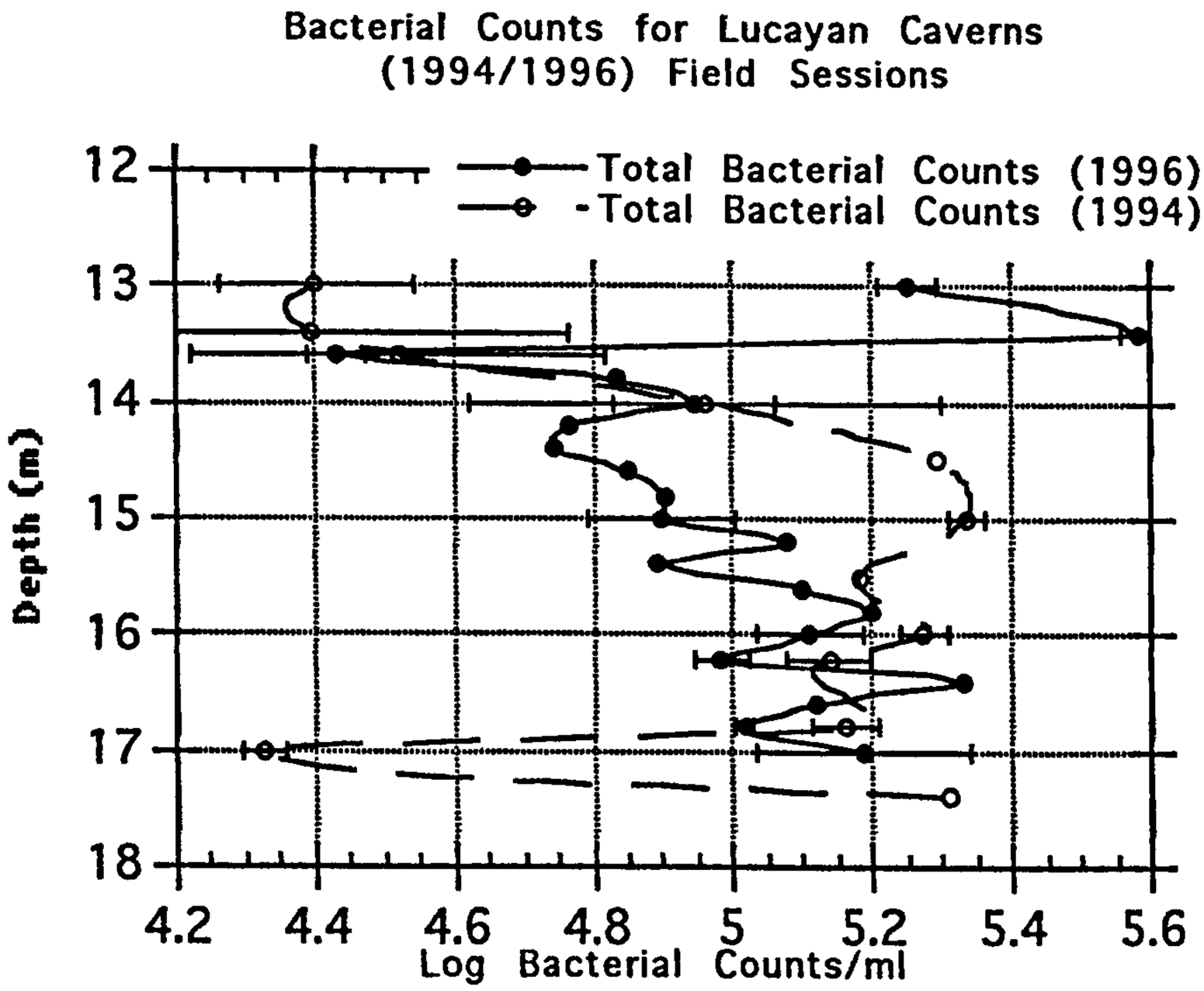


Figure 5.10: Total bacterial counts for 1994 and 1996 for the Lucayan Caverns. The bars represent (STD)

Bacterial numbers increased rapidly through the UMB and cell counts continued to increase with depth. Compared to the 1994 results, the 1996 cell numbers increased continuously with depth whereas in the 1994 data group, cell numbers increased from the fresh water body through the UMB and stabilized throughout the MMZ. However, once through the LMB, the cell numbers dropped before increasing sharply again within the marine section of the water column.

5:3:2 Dividing Cells

Results for the 1996 percent dividing cells (Figure 5.11) were slightly different from the 1994 results. In 1996 results show that percent dividing cells were the highest at 13.0 m, opposite the 1994 results, and from this depth vertically downward, the dividing cell numbers continued to decrease with a small increase within the MMZ at 15.8 m, just above the LMB. Comparing the 1994 results with the 1996 results the numbers throughout the water column stayed the same except that in 1996 large peaks occurred at 14.5 m within the MMZ and another peak at 17.4 m within the marine zone.

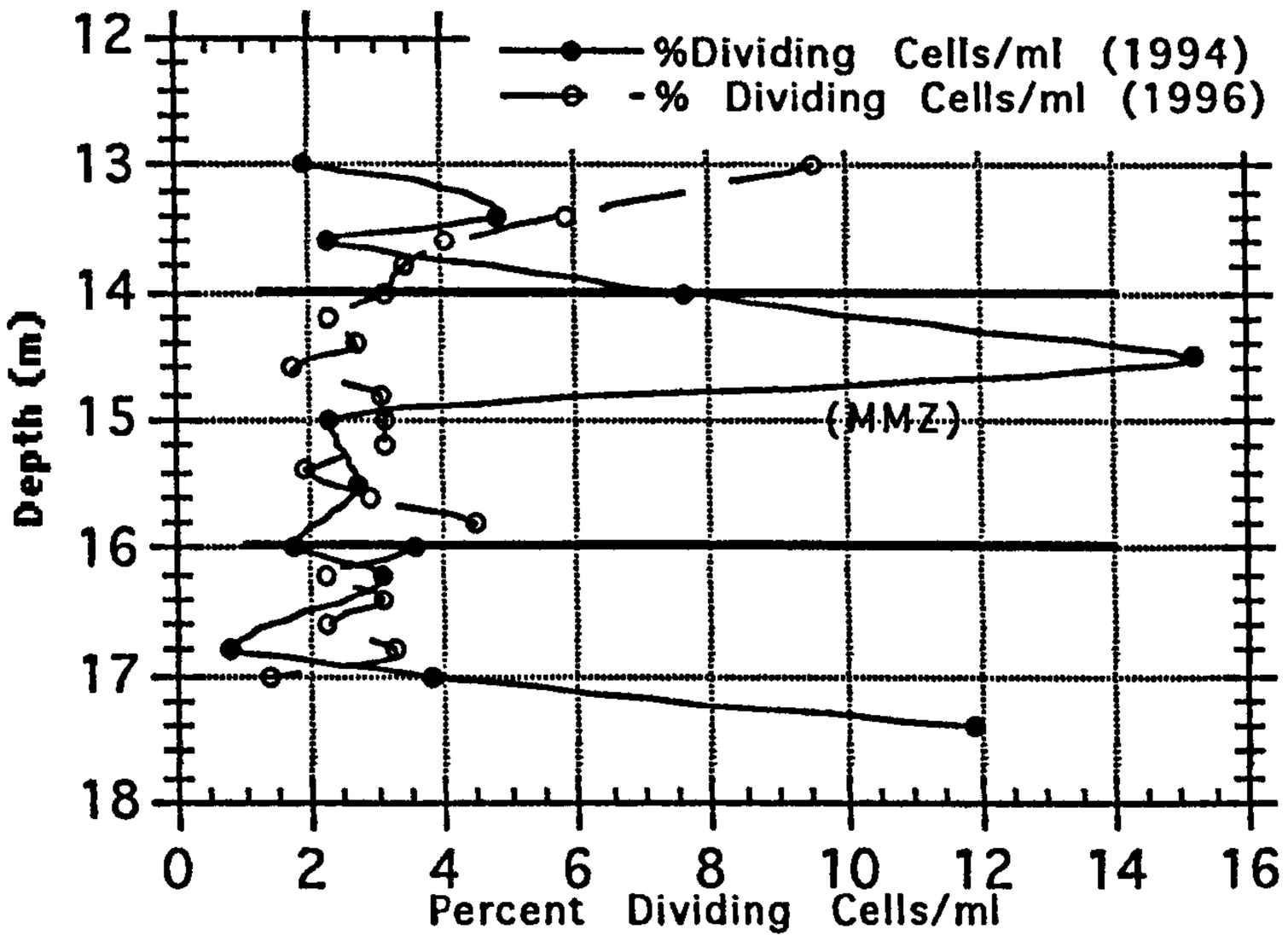


Figure 5.11: 1996 Lucayan Caverns Percent dividing cells results plotted against 1994 Lucayan Caverns results.

Overall, the 1996 results do not show that any significant region of the water column favors cell division.

5:3:3 Bacterial Counts on Particles

The 1996 and 1994 bacteria-on-particle populations (Figure 5.12) are quite different. The 1994 data was very variable and distribution did not seem to reflect the position of the major density gradients. The 1996 results are, in contrast, less erratic, and show a slight increase at the UMB, and

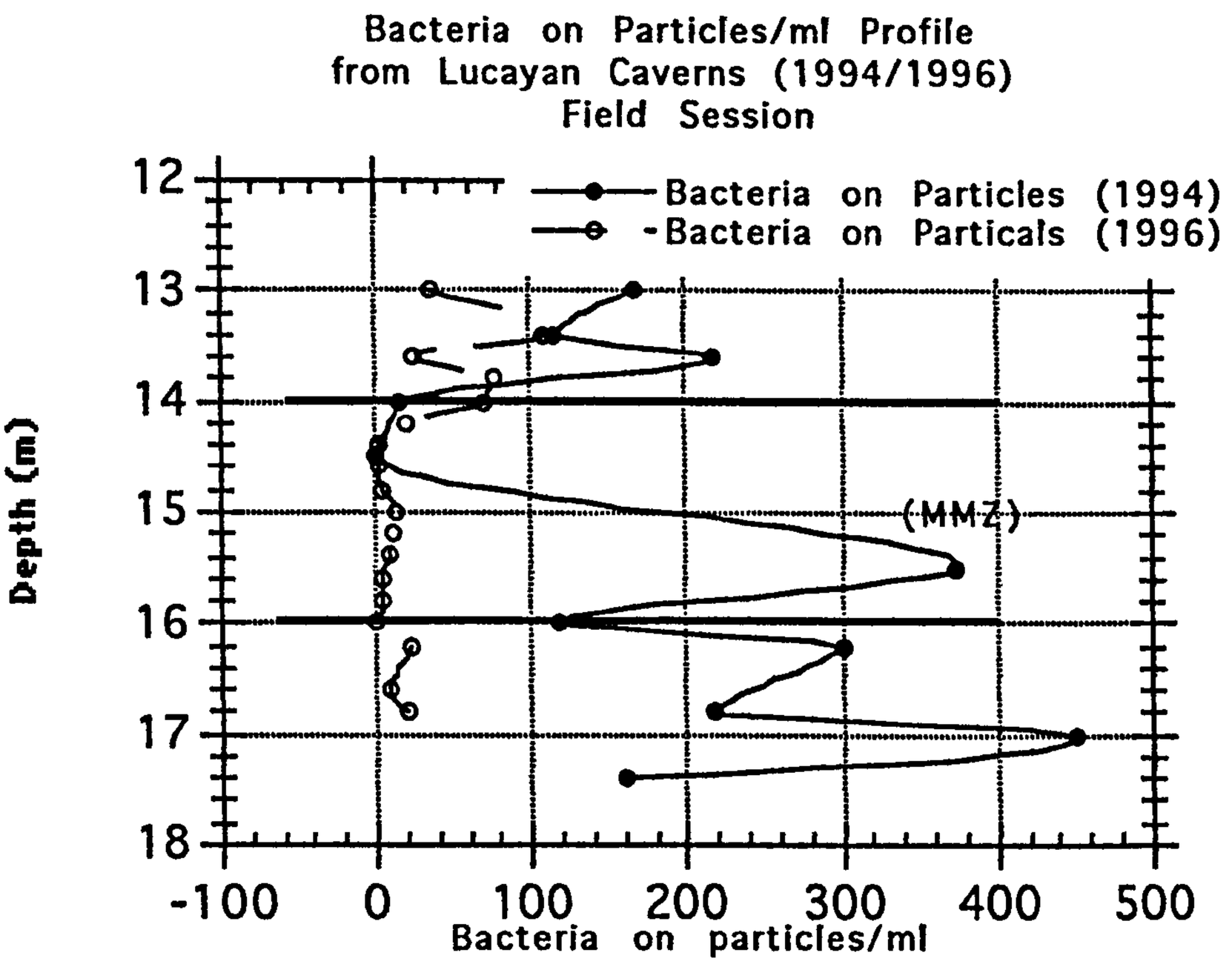


Figure 5.12: Bacteria-on-particles, 1996 plotted against the 1994 Lucayan Caverns results

below this population the numbers remain low throughout the MMZ with a slight increase below the LMB which also occurred in 1994. Plotting 1996 particulate organic matter against bacteria-on-particle (Figure 5.13) the results look different.

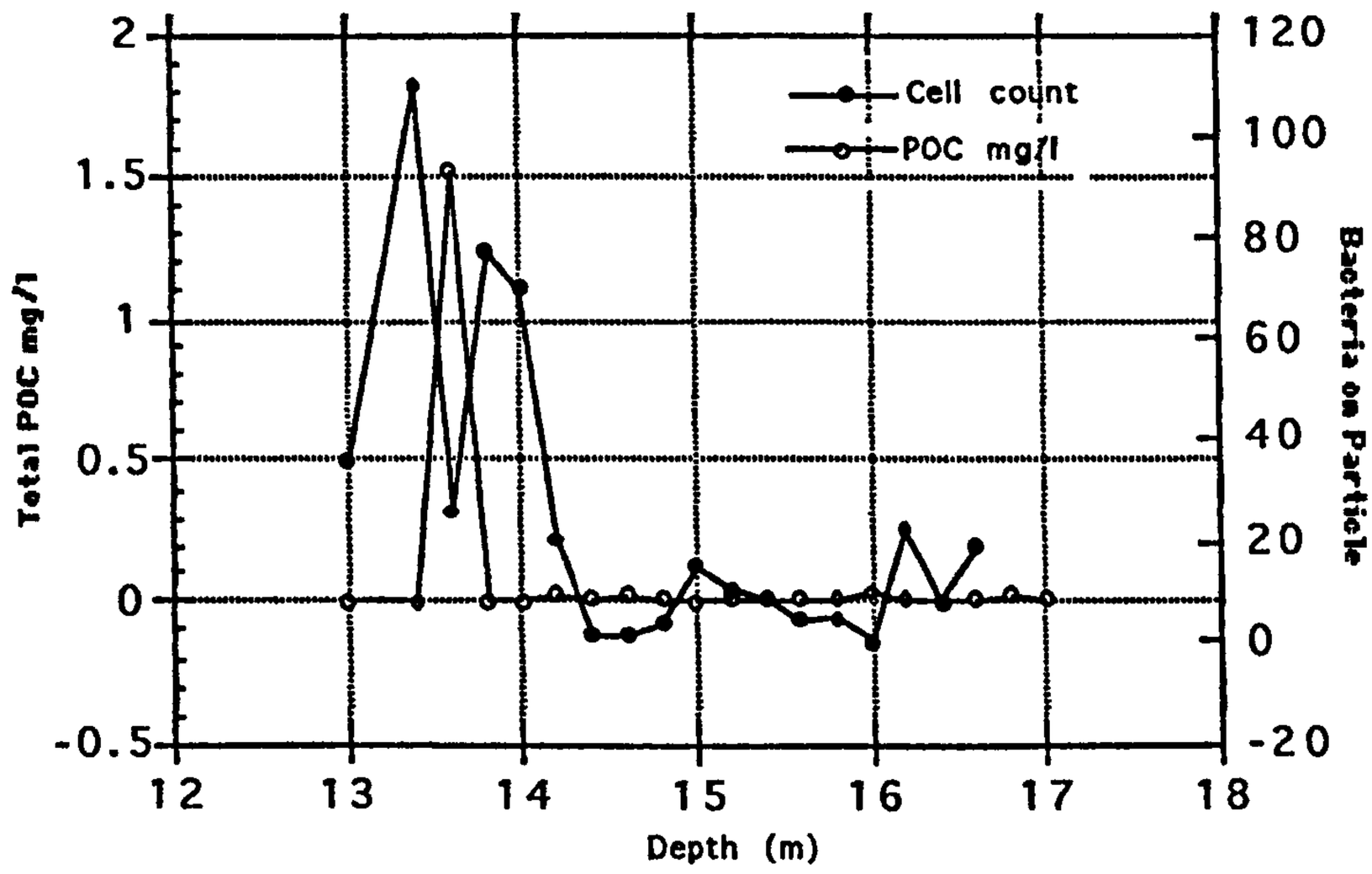


Figure 5.13: POC plotted against bacteria on particle; 1996 data.

5:3:4 Bacterial Counts off Particles

Although similar in numbers the 1996 bacteria-off-particle distribution (Figure 5.14) is almost the opposite of that of 1994. Where there is a low peak at 14.0 m in the 1994 data, the 1996 results show a high peak. Overall, the bacteria-off-particle results show bacterial numbers to be

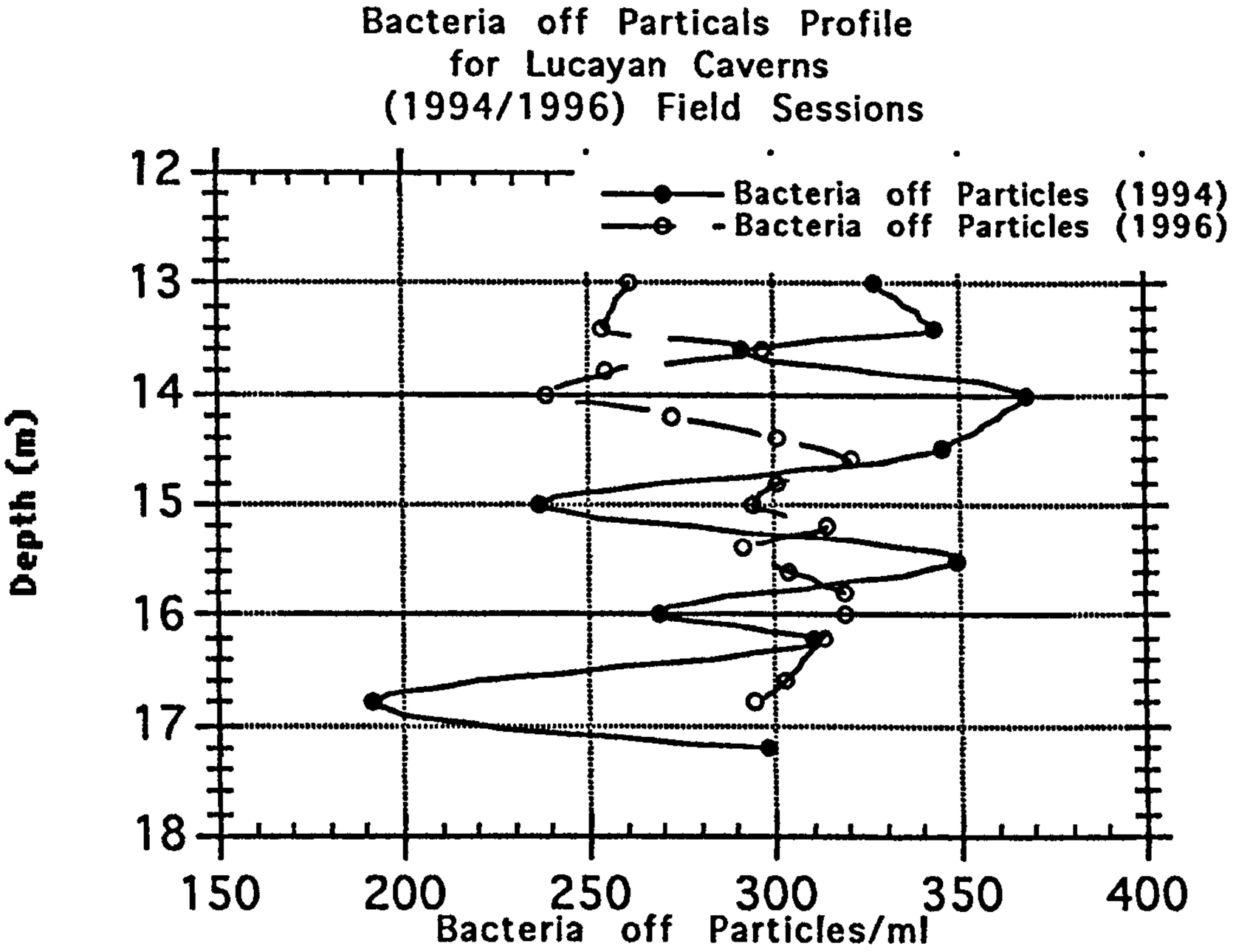


Figure 5.14: Lucayan Caverns results for Wedding Hall bacteria-off-particle counts

increasing with depth whereas in the 1994 results, the opposite was occurring. Neither result appears to reflect geochemical changes occurring with depth in the vertical water column.

5:4 Geology

5:4:1 Wall Rock Total Bacterial Counts

Bacterial populations from water samples collected at 13 m, 14 m, and 15.9 m, are only slightly higher than the bacterial numbers found at 8 cm into the wall rock at corresponding depths (Figure 5.15). The highest number of bacteria in rock sample was found from the surface to 2 cm into the wall at all three depths (Figure 5.16). At consecutive depths into the wall, the bacterial numbers slowly decreased.

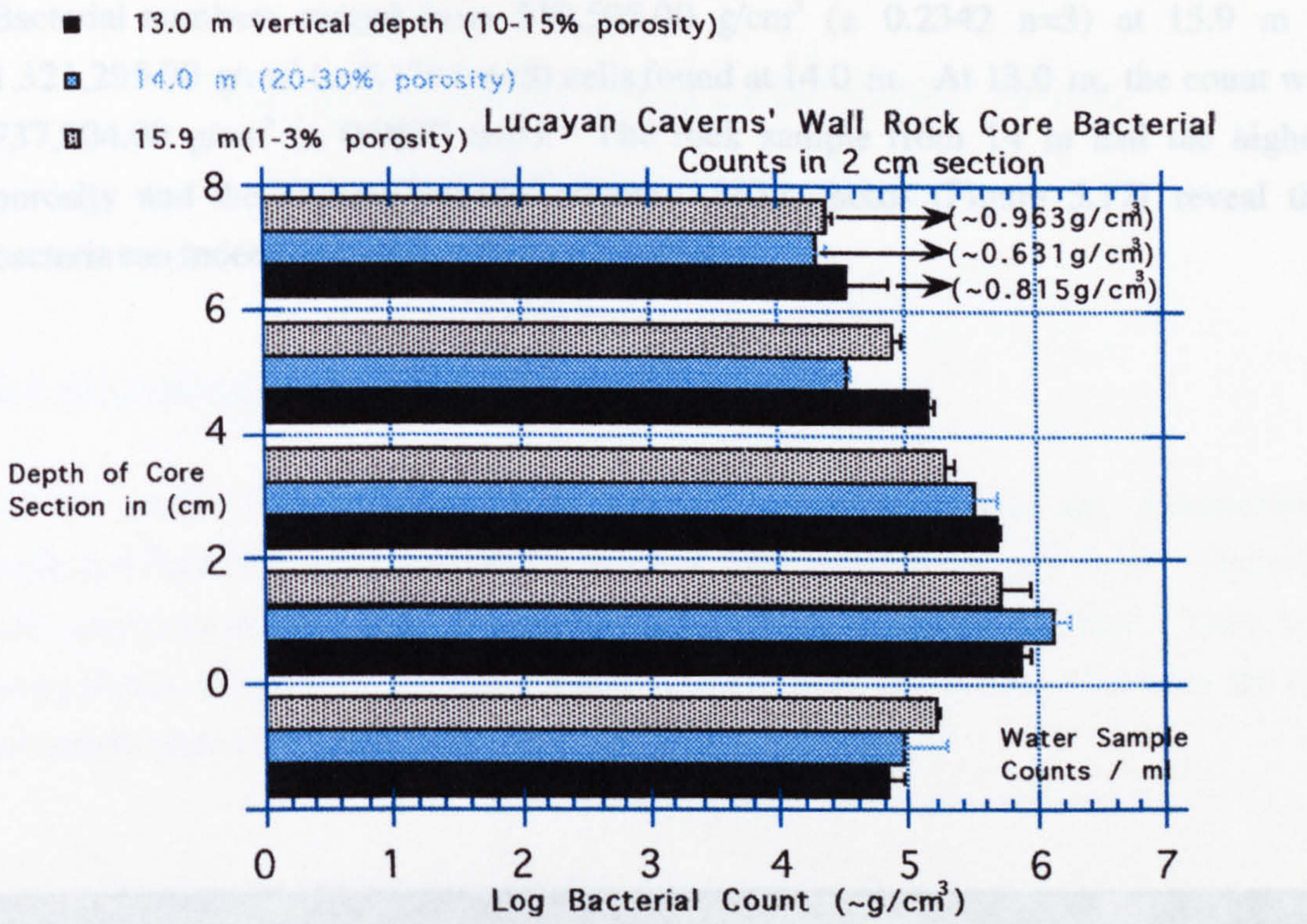


Figure 5.15: Total bacterial populations from rock cores from different water depths sliced into 2.0 cm sections and compared against populations in the water at the same depth.

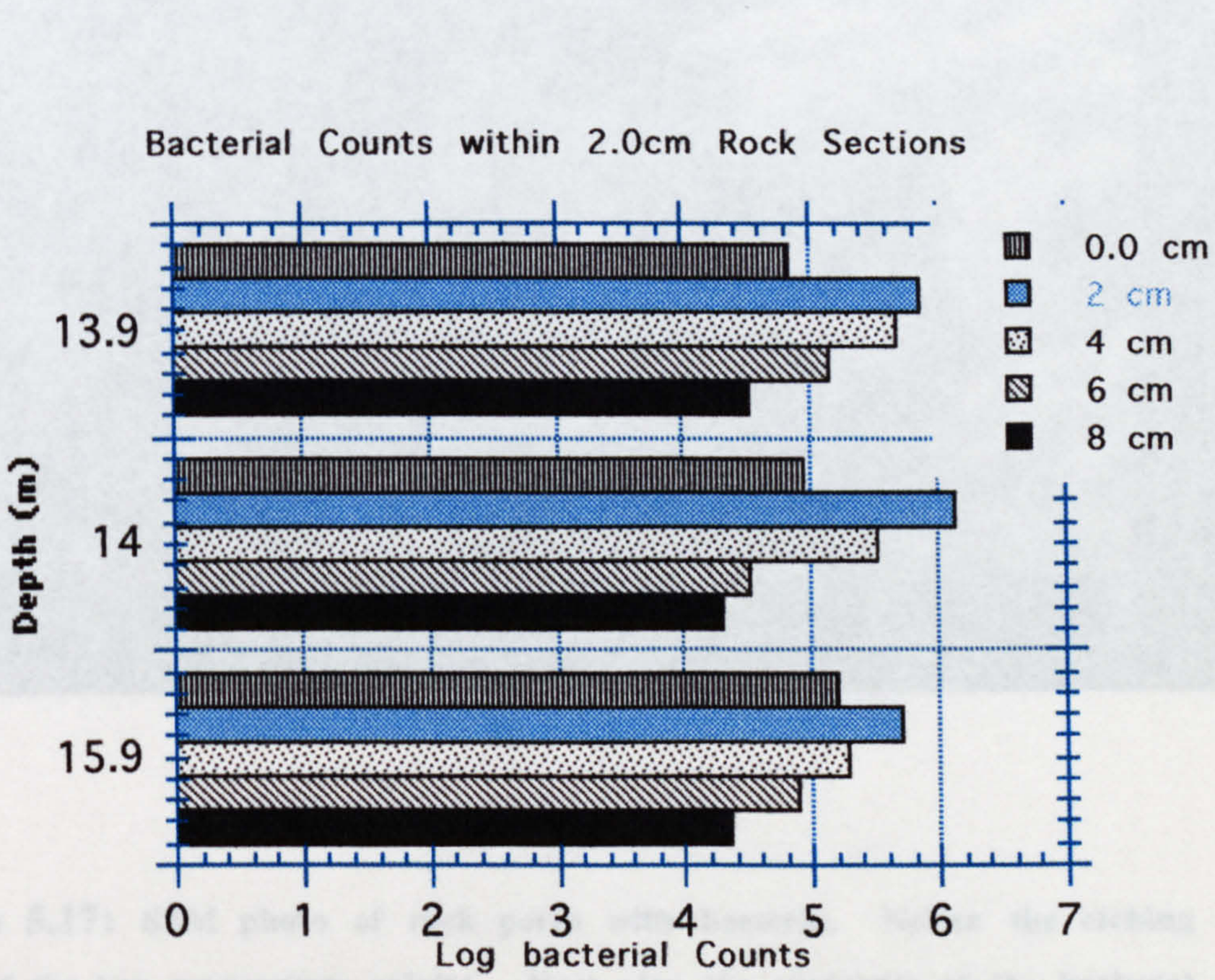


Figure 5.16: Bacterial populations with depth into wall rock showing consistent elevation of populations at all three water depths.

Bacterial numbers ranged from 510,505.00 g/cm^3 (± 0.2342 n=3) at 15.9 m to 1,321,295.00 g/cm^3 (± 0.1361 n=3) cells found at 14.0 m. At 13.0 m, the count was 737,904.00 g/cm^3 (± 0.0687 n=3). The rock sample from 14 m had the highest porosity and the highest bacterial counts. SEM photos (Figure 5.17) reveal that bacteria can indeed be found within rock samples.

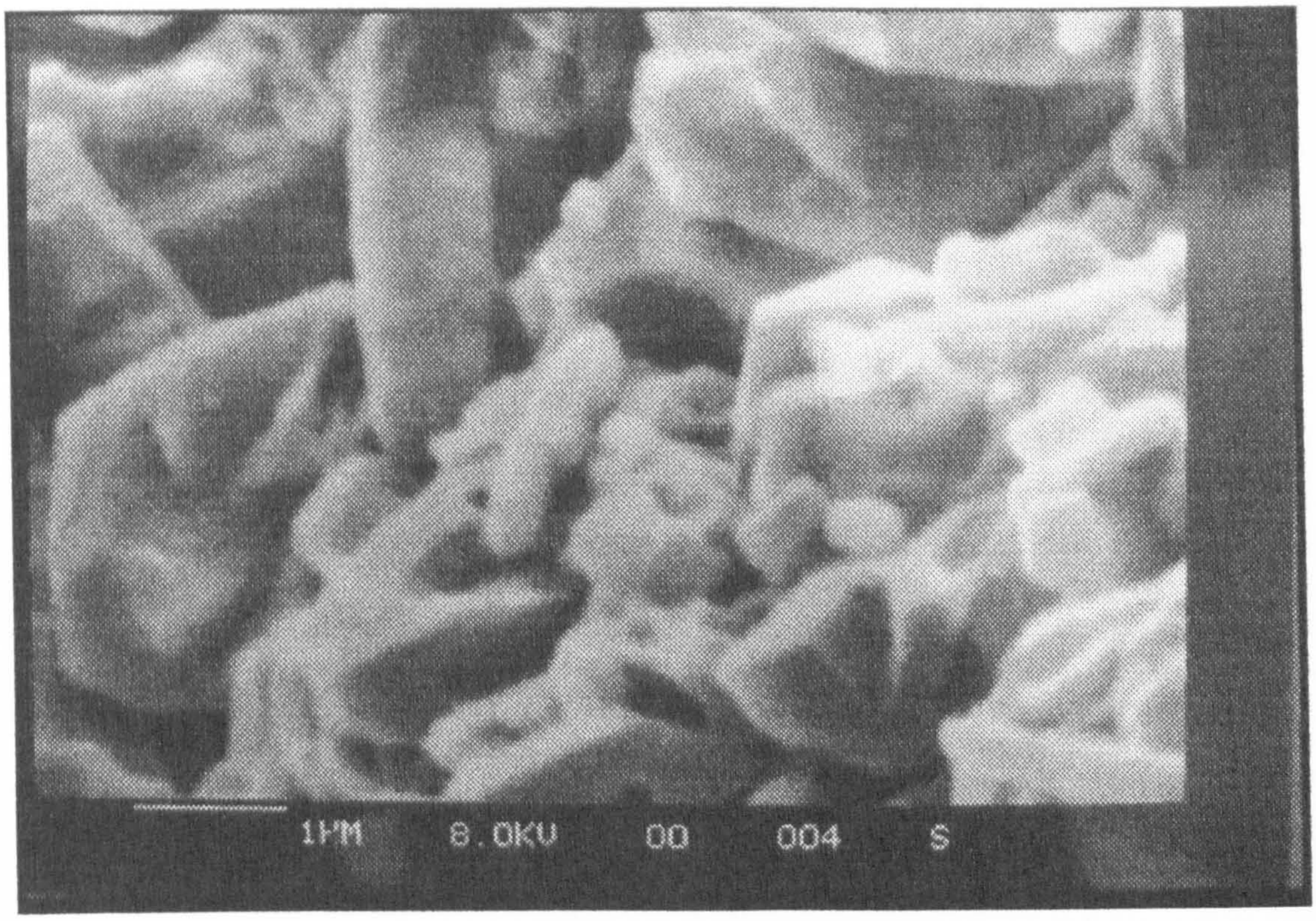


Figure 5.17: SEM photo of rock pores with bacteria. Notice the etching and pores of the low magnesium calcite. Note also the curvature of the bacterial cell wall in the centre of the photomicrograph.

Samples from Stargate Blue Hole, South Andros Island

5:5 Geochemistry

All the results from Stargate 1996, which are described here, are measurements collected from the south passage within the cave system (Figure 2.4). Hydrolab measurements in 1998, however, came from the vertical entrance shaft. They have been plotted with corresponding data for comparison. The distance between the two measured sites is approximately 100 m.

5:5:1 Salinity

The water column at this site, similar to earlier described sites, can be divided into three main bodies of water according to salinity: 1) fresh water body from 0 m to 15.49 m, 2) MMZ from 15.5 m to 26 m and 3) the marine section from 26.1 m onward to depth. The UMB is found at 15.5 m and the LMB at 26 m. As mentioned in earlier salinity profile descriptions, the definition of biologically fresh water is water having a maximum salinity of 0.5 g/l. For this study, again, the body of water from 15.49 m to 0 m will be considered the fresh water body although the water, even at sea level, is biologically nowhere near being fresh.

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5:5:1 Salinity

The water column at this site, similar to earlier described sites, can be divided into three main bodies of water according to salinity: 1) fresh water body from 0 m to 15.49 m, 2) MMZ from 15.5 m to 26 m and 3) the marine section from 26.1 m onward to depth. The UMB is found at 15.5 m and the LMB at 26 m. As mentioned in earlier salinity profile descriptions, the definition of biologically fresh water is water having a maximum salinity of 0.5 g/l. For this study, again, the body of water from 15.49 m to 0 m will be considered the fresh water body although the water, even at sea level, is biologically nowhere near being fresh.

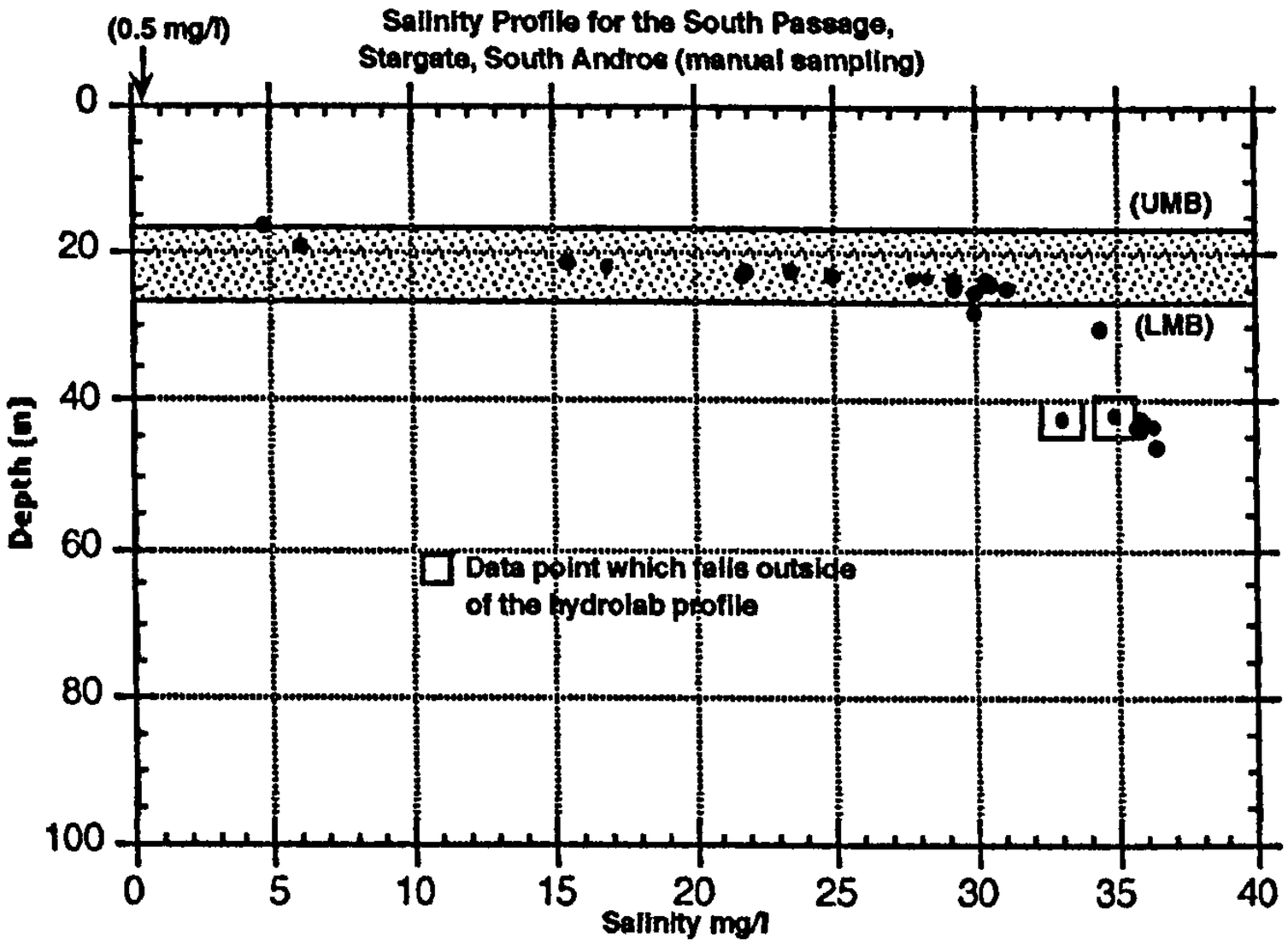


Figure 5.18 **Salinity profile for Stargate Blue Hole, South Andros. These measurements were taken in the south passage (refer to figure 2.4)**

Comparatively, the hydrolab profile for salinity (Figure 5.19) is almost identical to the salinity profile depicted on Figure 5.18. Only two data points, outlined with a box, fall slightly outside of the hydrolab profile. The double line of data points is a result of the hydrolab being lowered and raised again. It is possible to remove the return data points but from a point of interest, the data points were left in. Measurements for the hydrolab profile took a total of 15.6 minutes, one measurement every second.

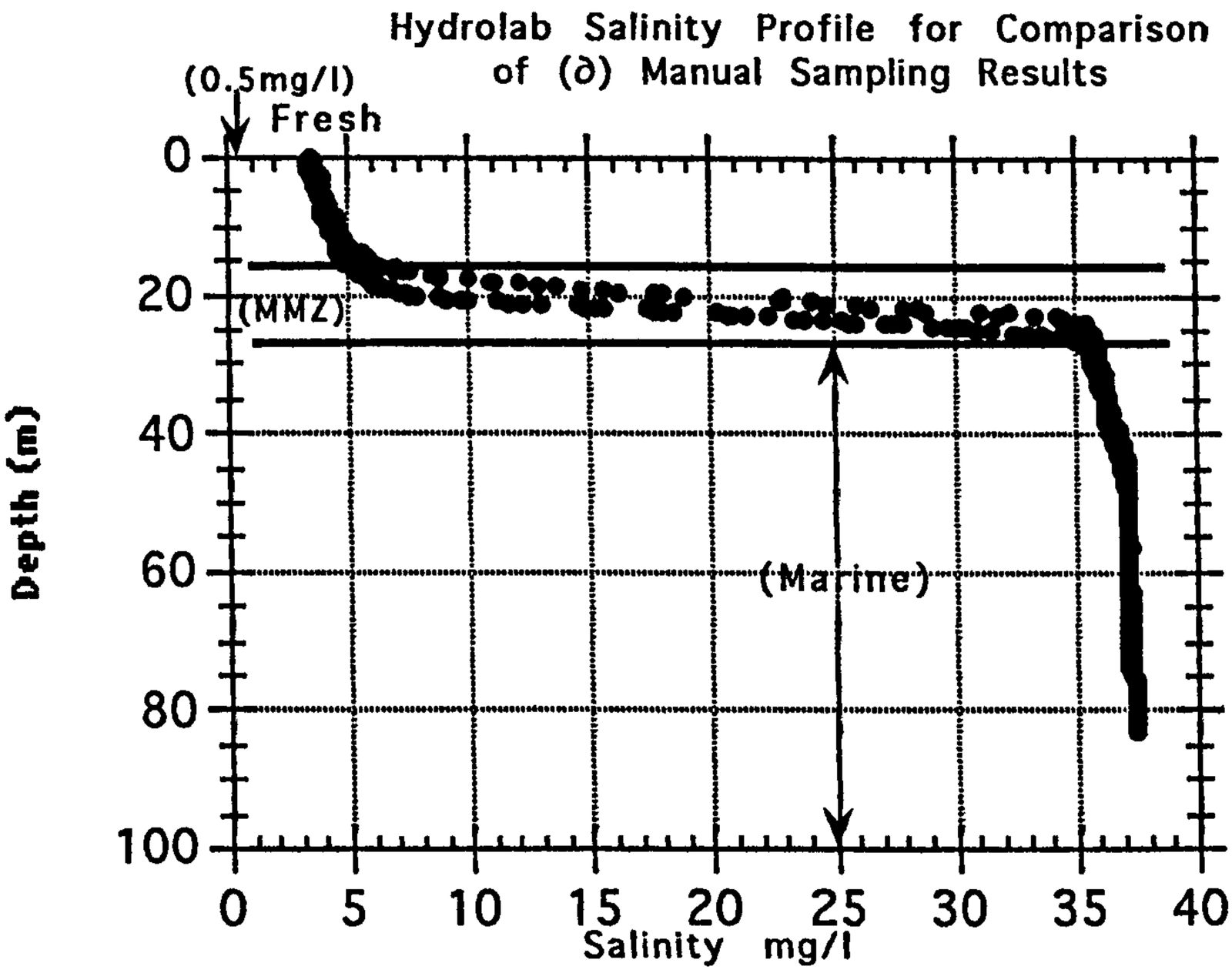


Figure 5.19 **Hydrolab profile for salinity. These measurements were taken in the entrance shaft of Stargate. This profile has 936 data points, 468 points for the profile going down into the shaft and 468 coming back up the shaft. Measurements were taken every second, needing a total of 15.6 minutes to be completed.**

Whitaker, (1992) collected water samples in 1986 and 1987 within the entrance of Stargate Blue Hole. Instead of plotting salinity Cl measurements were used (Figure 5.20). The collective data presented here in this section represent a time span of 12 years, and over the 12 years the salinity profile within the entrance of Stargate Blue Hole, is

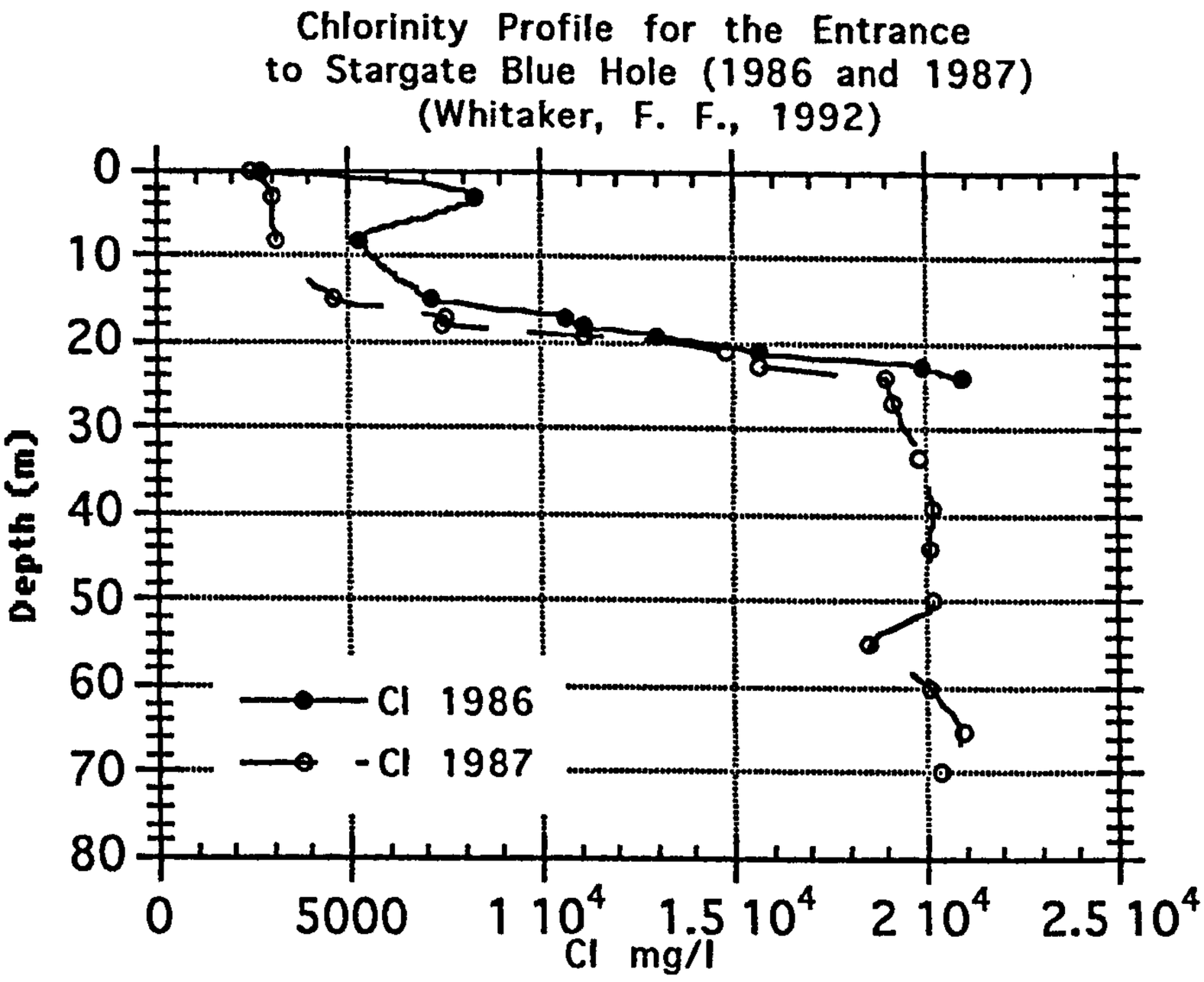


Figure 5.20: Whitaker 1986 and 1987 chlorinity profile results for the entrance of Stargate Blue Hole

basically unchanged. The UMB is at about 15.5 m and the LMB around 26 m, other geochemical perameters however have changed.

5:5:2 Temperature

Temperature measurements within the south passage (Figure 5.21) range from 23.1°C at 16.6 m to 25.5° C at 46 m. Shallow water samples are normally 2° to 3° C warmer, but in February, when these samples were being collected, the Bahamas experienced an unusual cold snap. Samples came out of the cave around midnight with air temperatures hovering around 4° C. It is very possible that some of the warmth

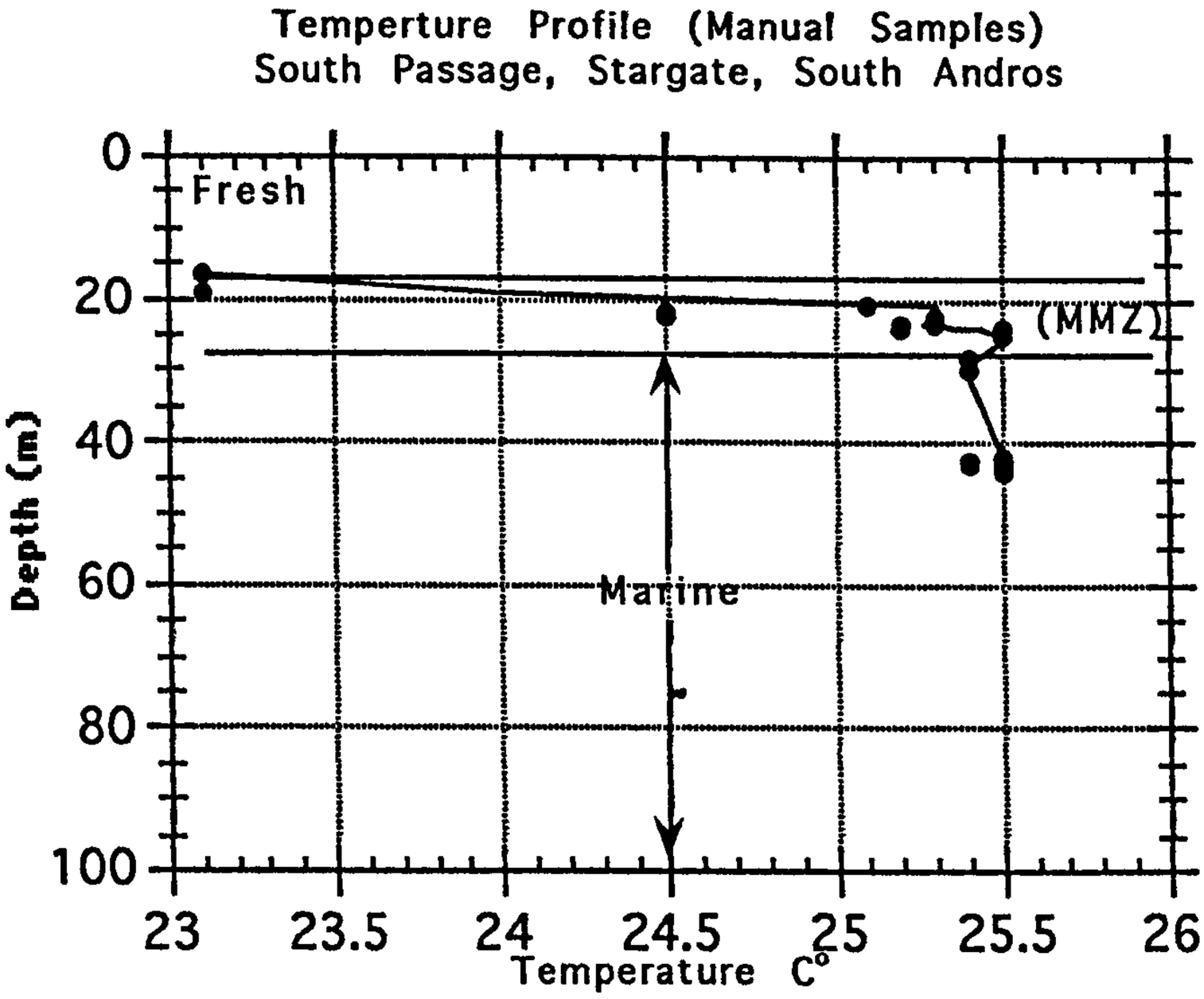


Figure 5.21 **Temperature recordings for the water column within the south passage in Stargate (measurements taken in February)**

of the samples was lost to the atmosphere following removal from the cave. The hydrolab temperature profile for comparison (Figure 5.22), which was recorded in August of 1997, demonstrates that the temperatures within the entrance shaft are much warmer at this time of year as expected. Although the fresh water sequence is missing from Figure 5.21, the general temperature trend, beginning at the UMB, is similar with a sharp increase in temperature throughout the MMZ. At 44 m, both the manual samples and hydrolab samples generated the same temperature recording of 25.5° C.

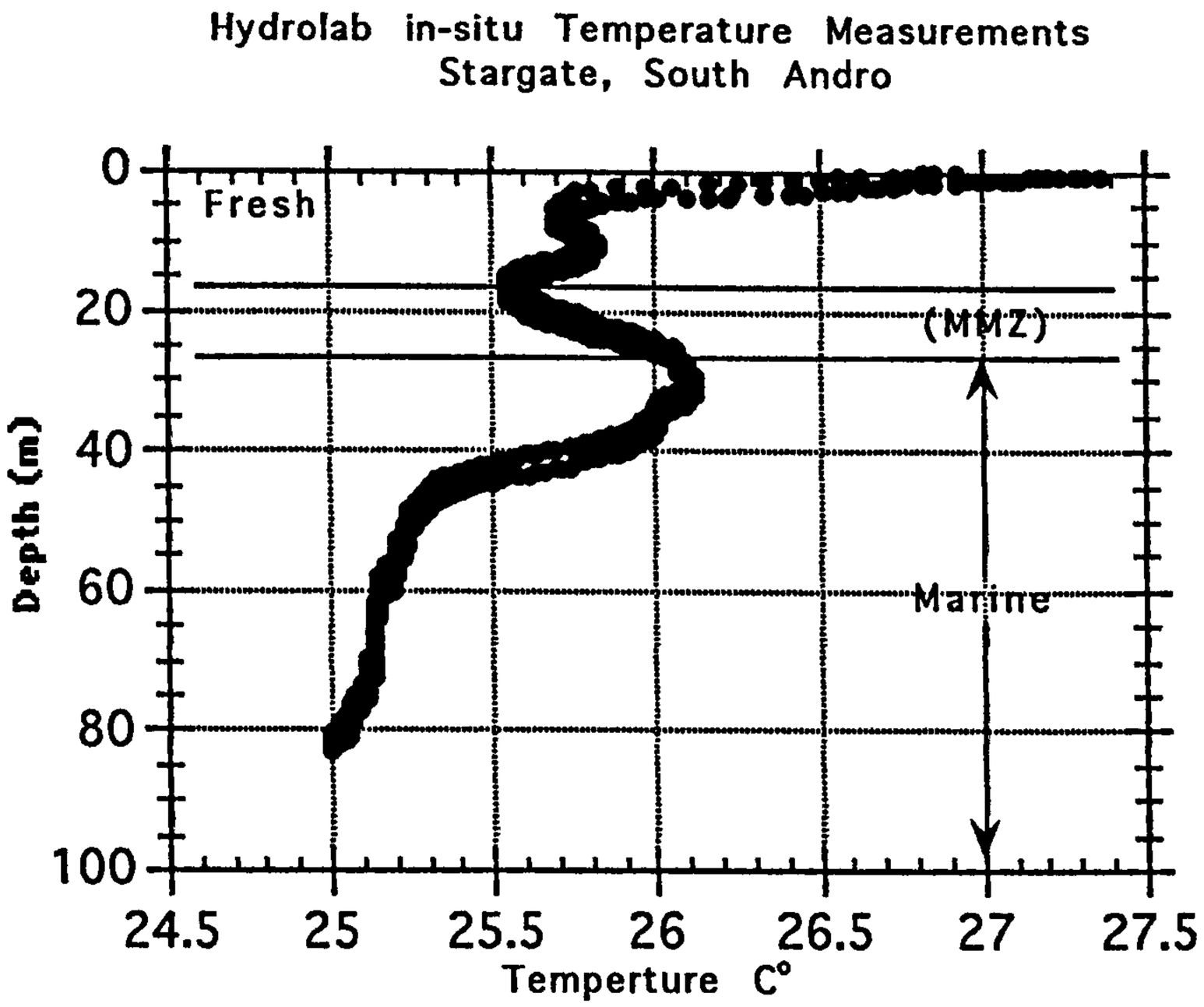


Figure 5.22 **Hydrolab temperature profile for the entrance shaft of Stargate Blue Hole (measurements taken in August). Double profile is for both measurements down to depth and return to surface measurements.**

Whitaker's temperature measurements for 1986/87 are slightly lower than the 1998 hydrolab measurements, and the temperatures of the surface waters from 1996 are significantly cooler than any of the other temperature profiles; it is postulated that results reflect seasonal temperature variations. The temperature trends, however, have not changed over the years or seasons. Temperatures are the warmest at the surface and cooler toward the UMB and, once through the UMB, are relatively stable but continue to cool with depth.

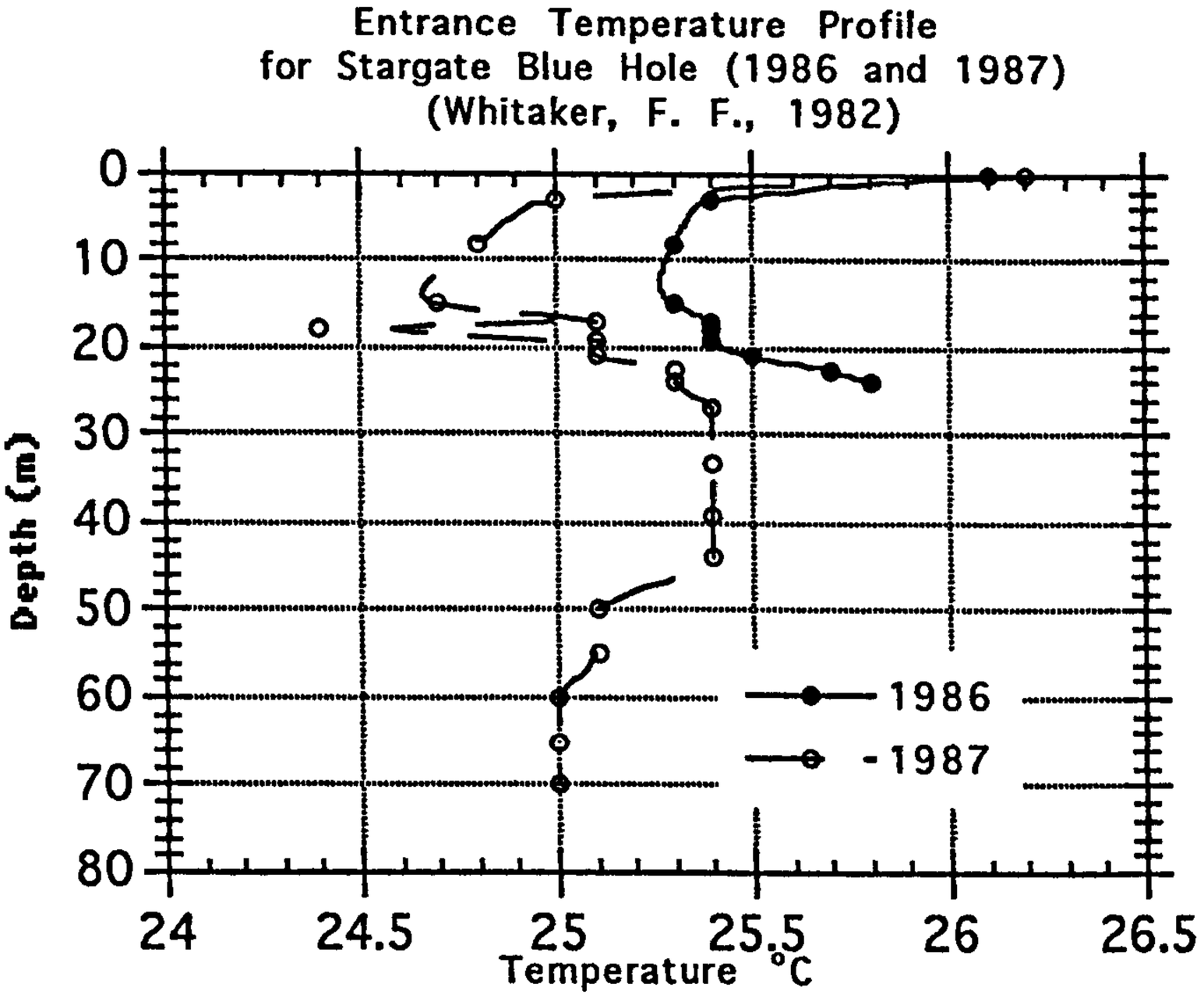


Figure 5.23: **Entrance temperature profile for Stargate Blue Hole (1986 measurements taken in July/August and 1987 measurements taken in September/October), (Whitaker, F. F., 1992)**

5:5:3 pH

The pH values (Figure 5.24) from the south passage of Stargate show high variations, however, hydrolab results confirmed previous measurements.

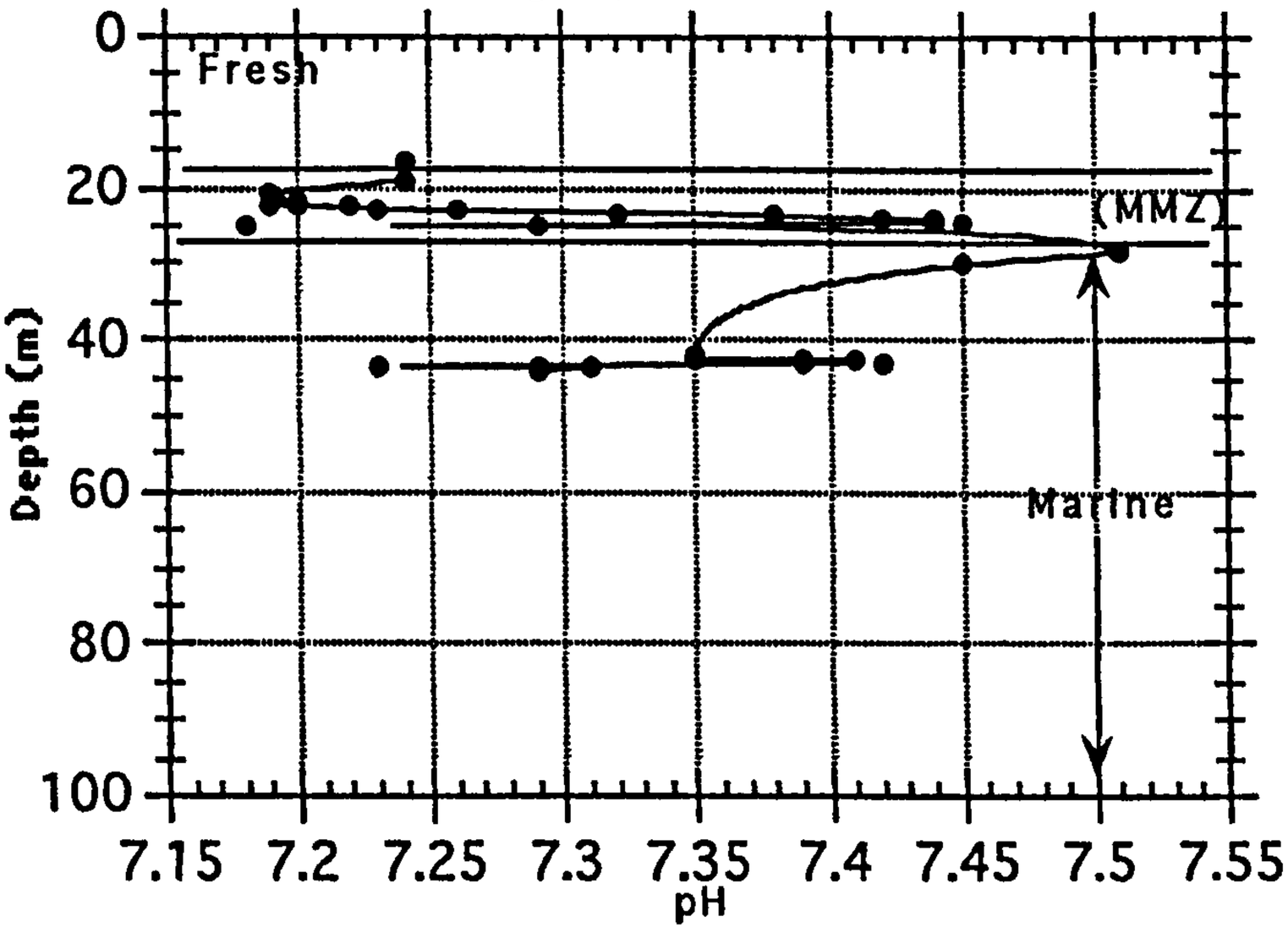


Figure 5.24: pH measurements for the south passage in Stargate Blue Hole

The pH values show that the entrance waters are much more alkaline than the waters located within the south passage. Similar shifts in pH values have been measured in the Lucayan Caverns (refer to fig. 4.16 D) and in the Owl's Hole (refer to fig. 4.42 D).

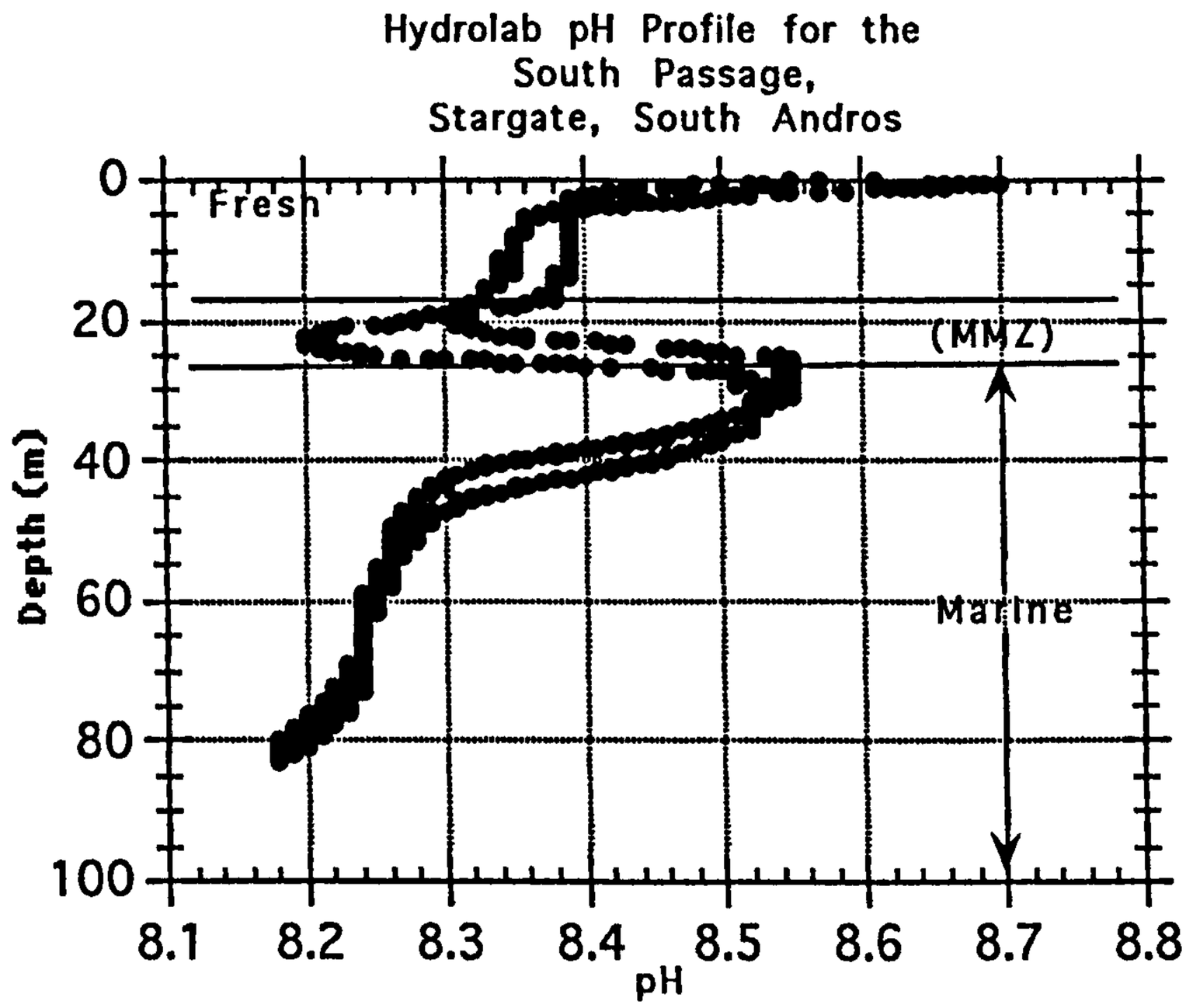


Figure 5.25: Hydrolab pH recordings for the entrance shaft in Stargate

In Figure 5.24, pH values decrease sharply once through the UMB and in the middle of the MMZ pH values become more alkaline; the same is seen in the hydrolab profile (Figure 5.25). This increase in pH values continues till the LMB is reached and from this point onward, pH values are more acidic.

Examining Whitaker's pH results from 1986/87, the entrance pH values are very different from the hydrolab results and more similar to 1996 results. However, the high pH values from the hydrolab are not unusual in that several other blue holes found along the same fracture system and sampled by Whitaker have the same pH range as the hydrolab results.

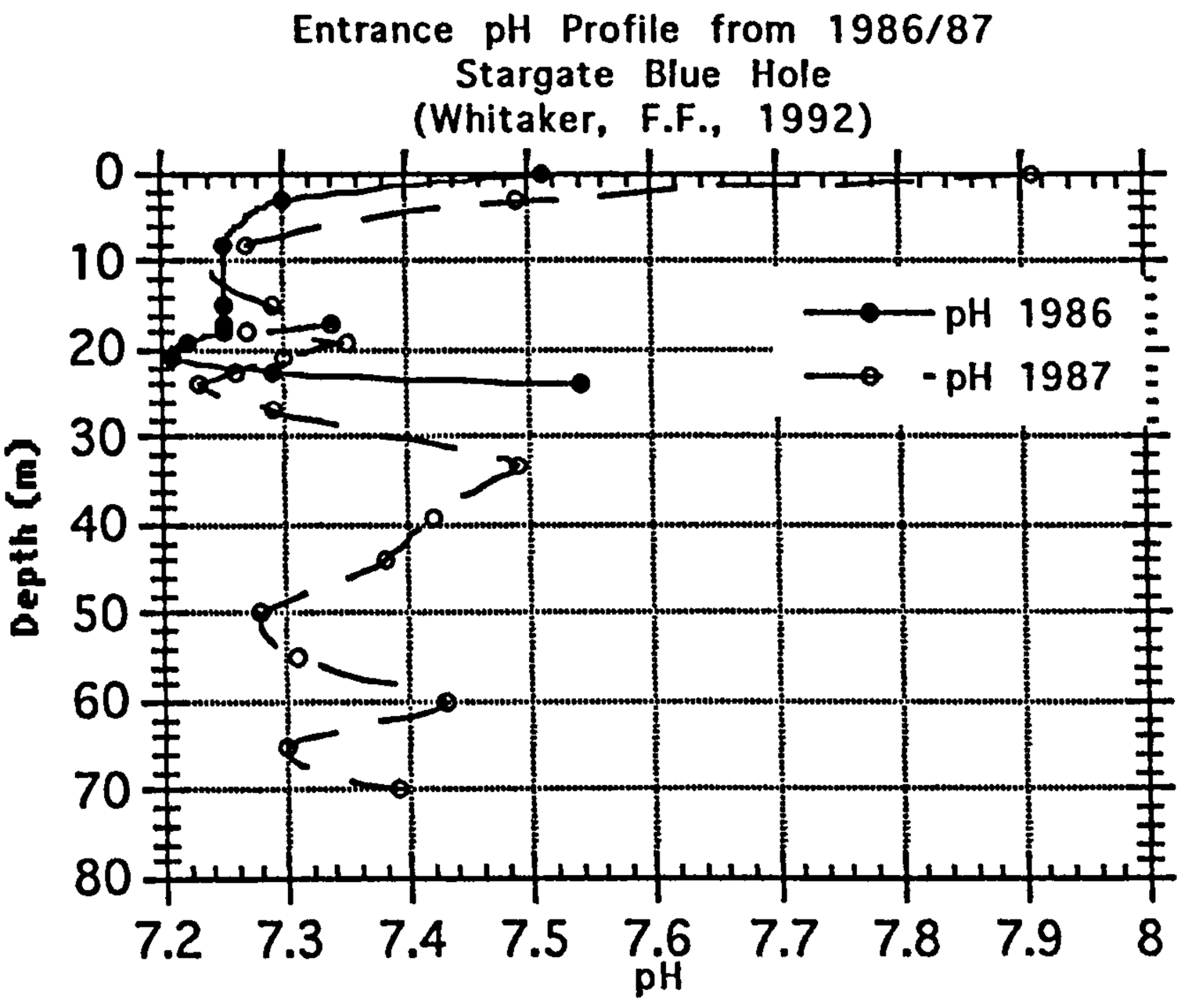


Figure 5.26: Entrance pH profile for the Stargate Blue Hole (1986 and 1987) (Whitaker, F. F., 1992)

5:5:4 Alkalinity

The 1996 alkalinity measurements are similiar to the entrance measurements taken by Whitaker (1986/87). As expected, alkalinity values decrease with depth and increased salinity.

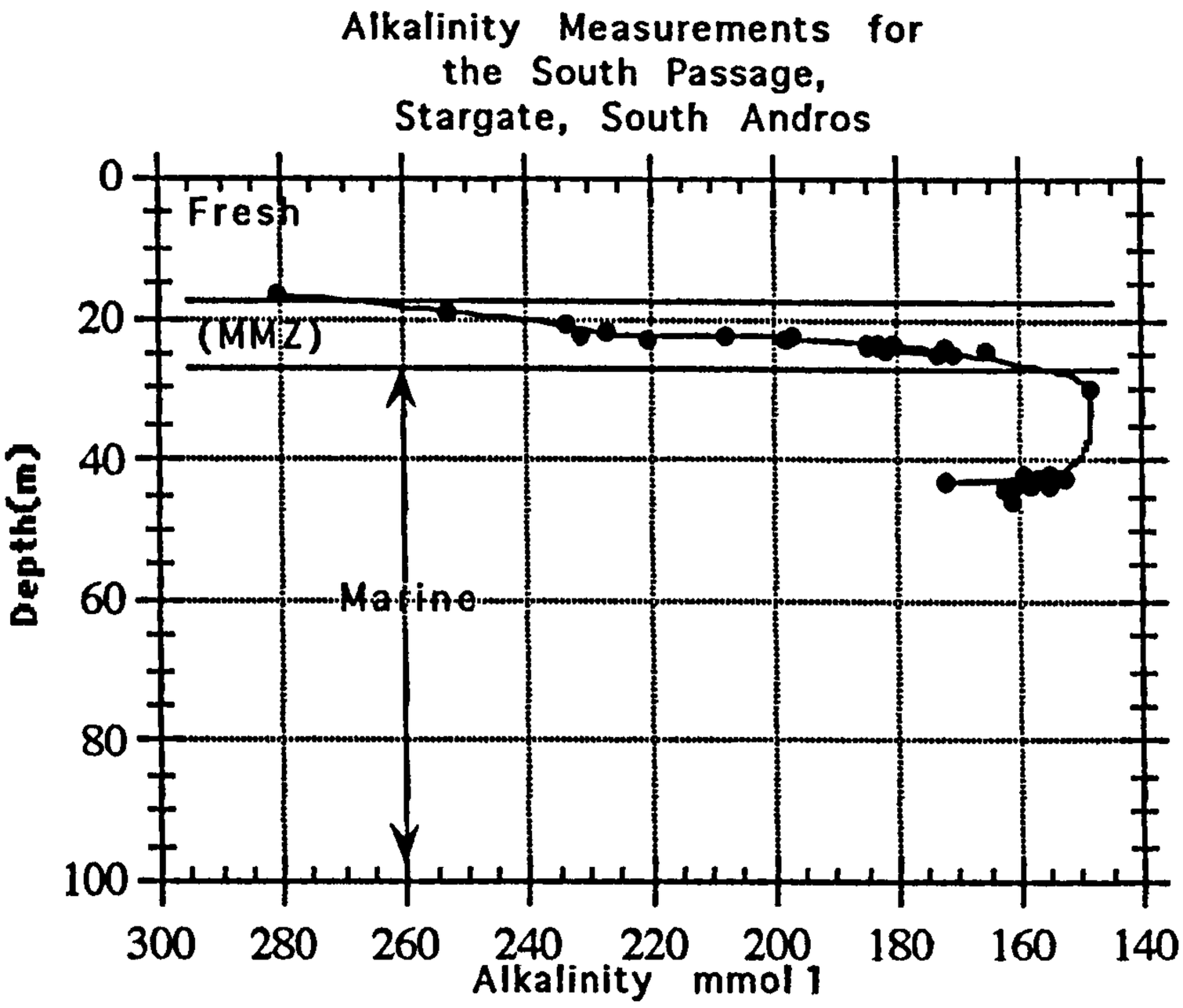


Figure 5.27: **Alkalinity results for the south passage in Stargate blue hole (1996)**

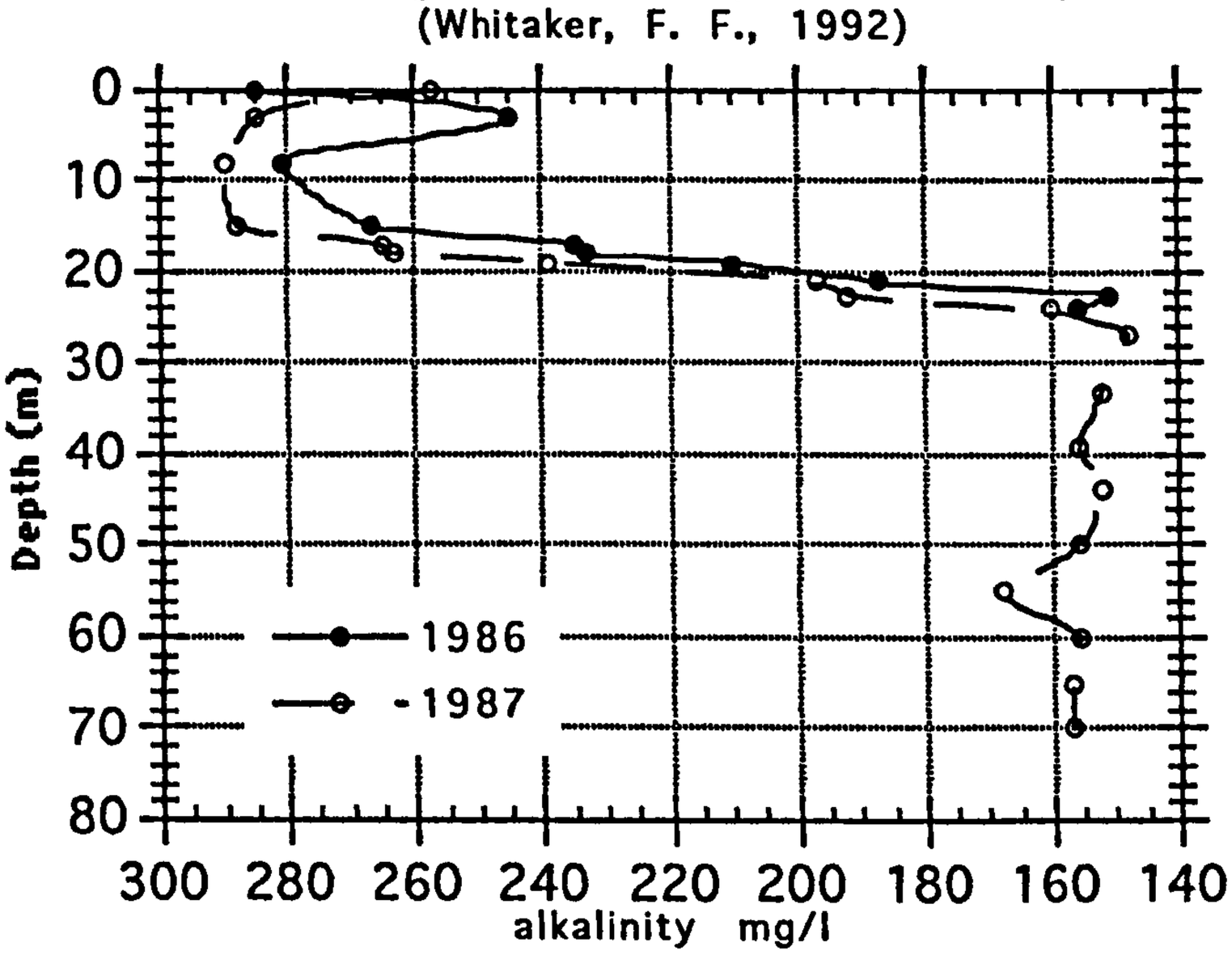


Figure 5.28: **Alkalinity results from the entrance of the Stargate blue hole (1986/87) (Whitaker, F. F., 1992)**

The fresh water section of the water column within the south passage is not represented here because this section of the water column was not accessible. The average alkalinity (Figure 5.27) for the MMZ was 201 $mmol^{-1}$. For the marine section of the water column the average was 157 $mmol^{-1}$.

Whitaker showed (1987) that the alkalinity average for the entrance to Stargate Blue Hole was 276 mg/l in the fresh water section and 219 mg/l in the MMZ. The marine section average was 155 mg/l.

5:5:5 Dissolved Oxygen

The DO profile for the south passage (Figure 5.29) shows a rapid decrease in DO levels through the MMZ. However, once through the LMB, DO levels increase over a few metres before continuing to decrease with depth. The DO measurements from below 40 m are suspect. It is possible that the DO probe was no longer working properly due to equipment failure or that H_2S concentrations may have rendered the DO probe useless. Similar problems arose with the hydrolab DO probe but the probe responded differently to the H_2S problem ((B) in Figure 5.30).

In examining the hydrolab DO profile, (Figure 5.30), the DO profile is similar to the upper section of the water column in the south passage. The DO profile within the entrance of Stargate Blue Hole registers absolute anoxic conditions at

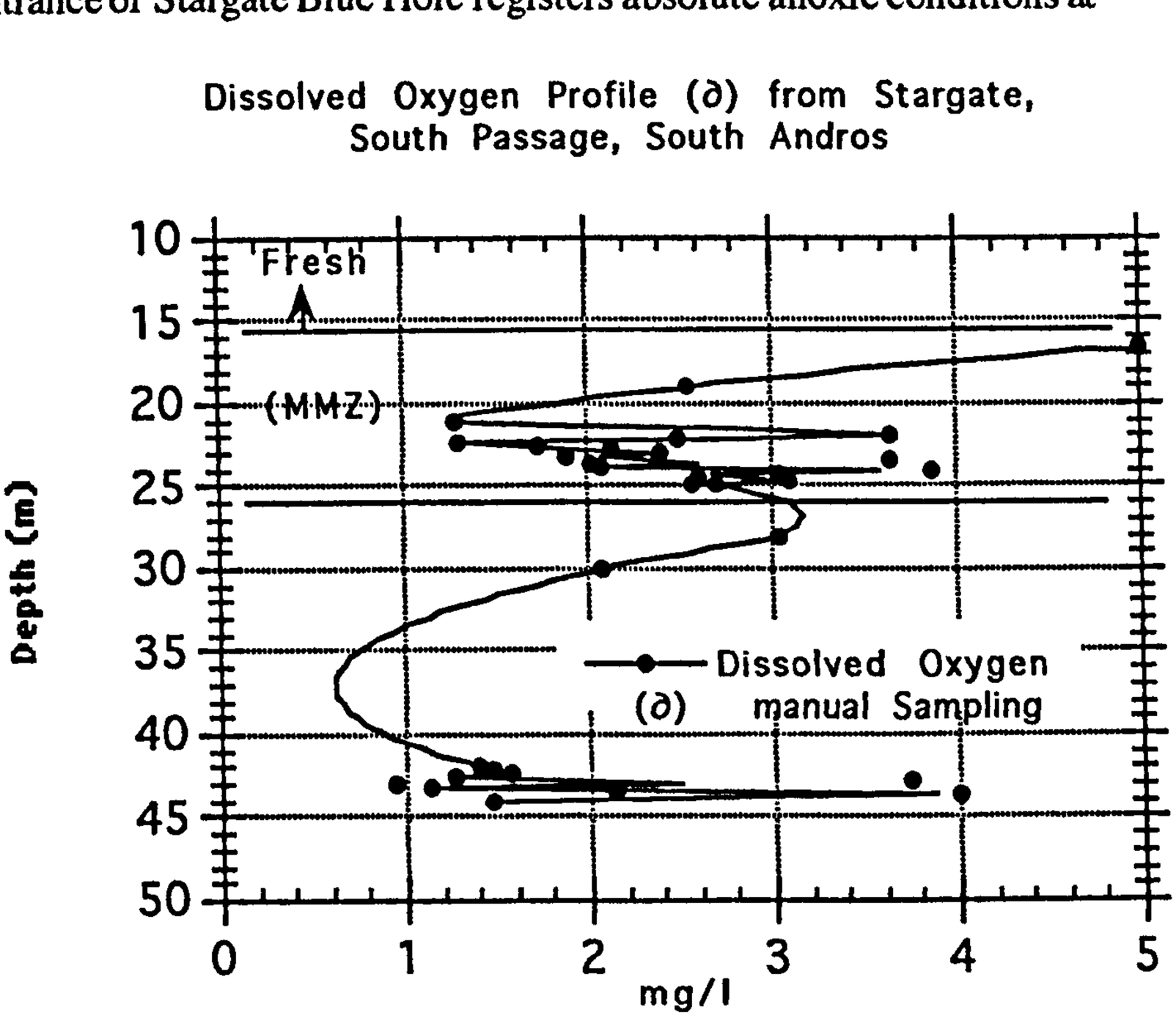


Figure 5.29: Dissolved oxygen profile for the south passage in Stargate Blue Hole (1996)

63.1 m (A) in (Figure 5.30), while 49.7 m is the first depth where DO values drop 0.9 mg/l. Another area of interest is directly below the LMB where there is a sudden short increase in DO. Although this feature is not as pronounced in the south passage as in the entrance shaft, the increase is real.

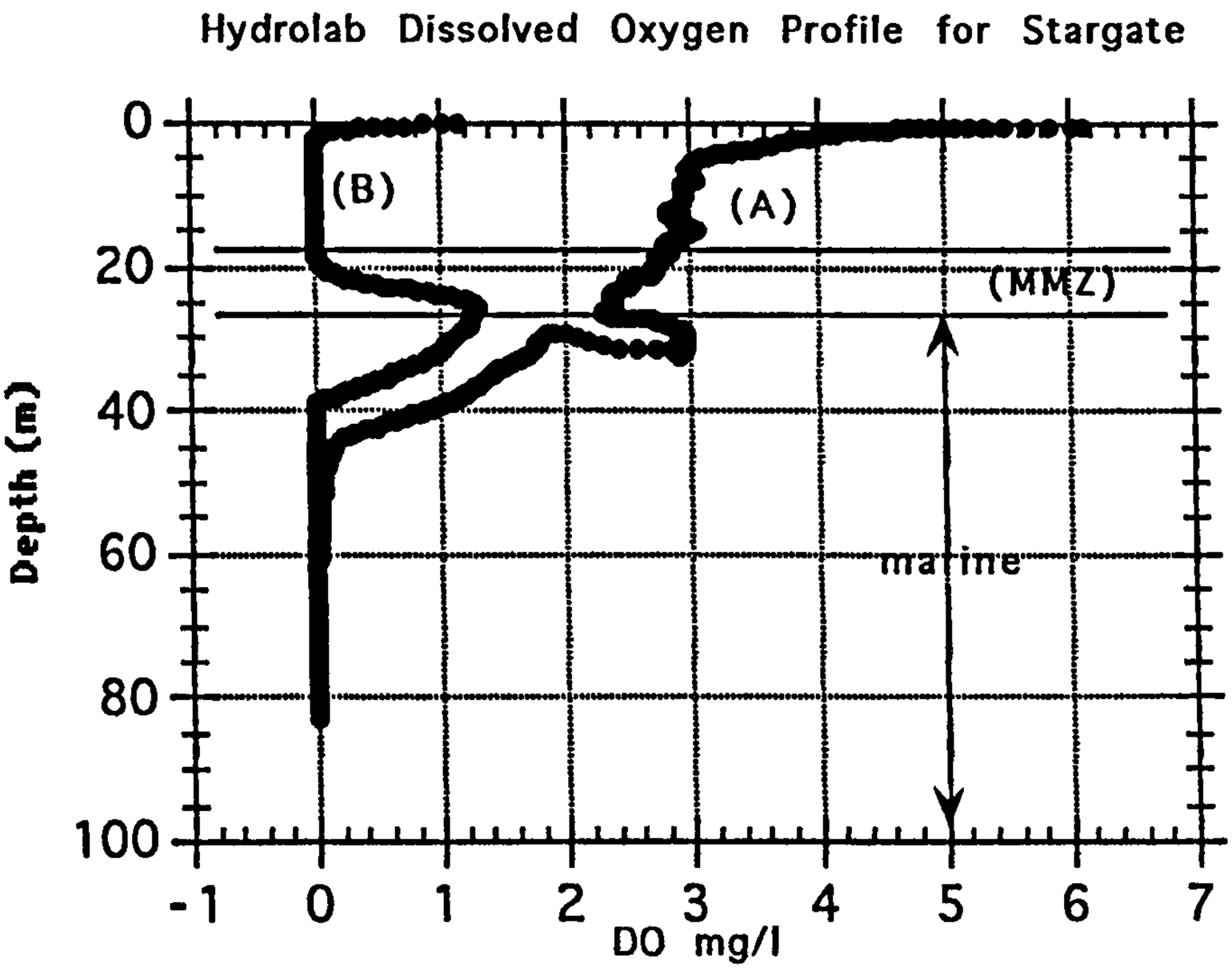


Figure 5.30: Hydrolab measurements of dissolved oxygen within the Stargate blue hole. Profile (A) is the probe beginning lowered through the water column. (B) is the probe returning from depth. The difference between the two has to do with temporary H_2S contamination of the O_2 membrane. (Total number of data points is 935; measurements were taken over a 15-minute period.)

5:5:6 Dissolved Organic Carbon

Stargate Blue Hole, because of its extensive water column, was sampled in regions where it appeared visually that there was a halocline and markings on the wall, indicating change in water chemistry. To sample the water column in its entirety would have required a great deal of supplies and additional man-power. Although there were three individuals involved in the data collection at this site the depth of the dives,

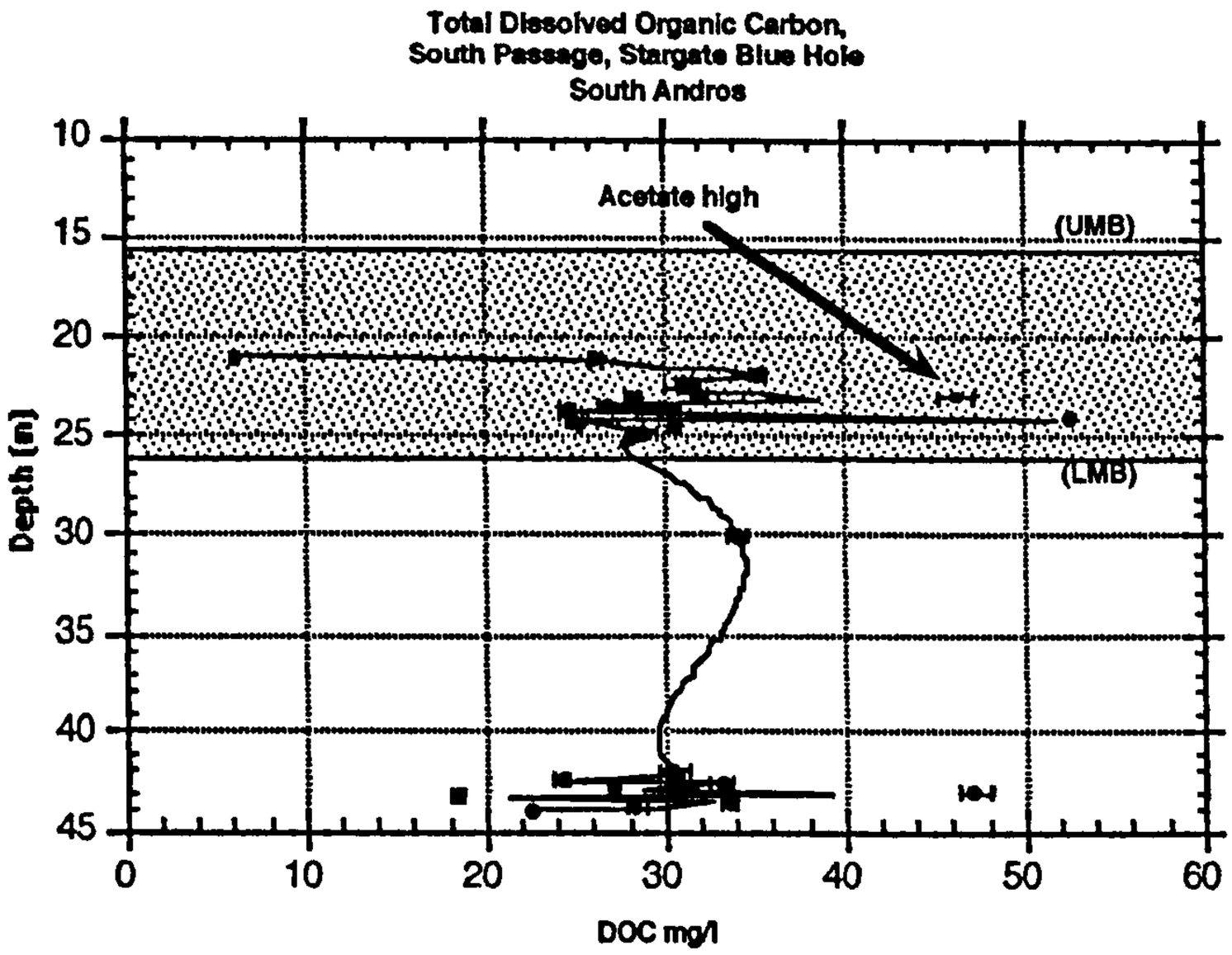


Figure 5.31: Dissolved organic carbon results for south passage, Stargate blue hole (1996). At 22 m is a peak which correlates with bioavailable acetate and also POC) (Figures 5.32; 5.33)

coupled with the necessary diving time, would have exposed the divers to the dangers of decompression sickness. For this reason data points are clustered and comparing the results may give a sense of more activity at a particular site than there really is.

The area of the water column which appears to have the most DOC is at 24 m with a measurement of 53 mg/l. The minimum value is at 15.5 m with a measurement of about 6.2 mg/l. Overall, the DOC amounts hover between 18 mg/l and 36 mg/l. These data were collected at the south passage in Stargate and not within the entrance shaft.

5:5:7 Particulate Organic Matter

The POM depth distributions (Figure 5.32) are broadly similar to that of DOC. The highest POM at 23 and 24 m coincide with the highest DOC. However, the lowest POM is found around the 25 m and 42 to 44 m regions within the lower mixing zone and marine zone respectively, in contrast to DOC (Figure 5.31), which was lowest at 15.5 m depth. The highest DOC values (refer to figure 5.31) are supported by the highest POM measurements taken at 23 to 24 m depth in the south passage of the

Stargate blue hole (Figure 5.32), whereas the lowest POM value was not at 15.5 m but in the area surrounding the 25 m and 42 to 44 m region within the water column.

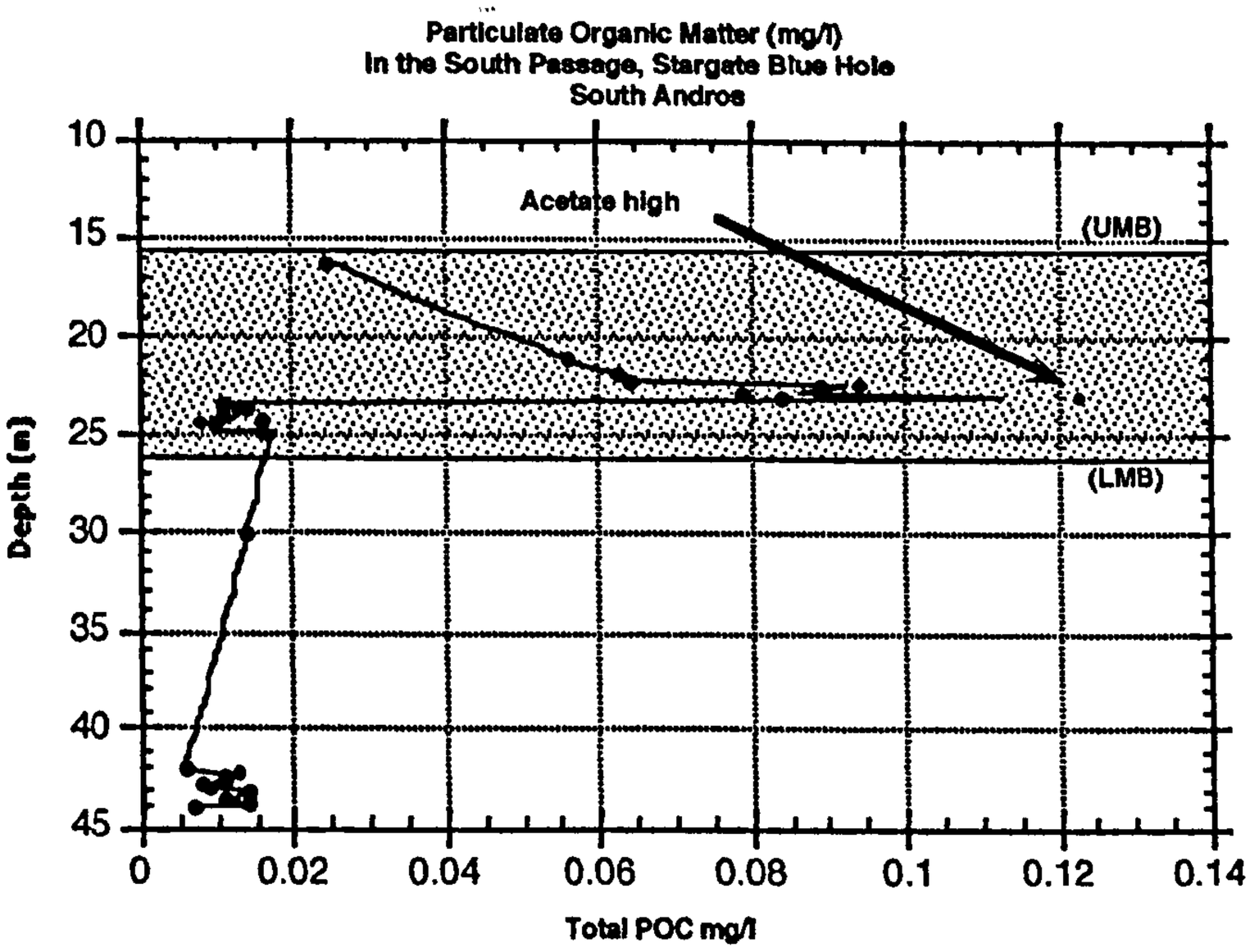


Figure 5.32: Total particulate organic matter found within samples collected in the south passage of Stargate Blue Hole. The arrow again points a peak which is found to correlate to high bioavaliable acetate measurements (Figure 5.33), high DOC (Figure 5.31)

Following the highest POM concentrations POM values decrease dramatically and appear to remain low throughout the rest of the water column following the LMB.

5:5:8 Acetate Concentrations

Even though the background measurements of acetate (Figure 5.33) within Stargate are not unlike the results found in the Lucayan Caverns and in Owl's Hole, the best comparison can be made with the Lucayan Caverns results. Maximum acetate concentrations of 29 μM occurred at and coincided with a zone of both high DOC (Figure 5.31) and POM (Figure 5.32).

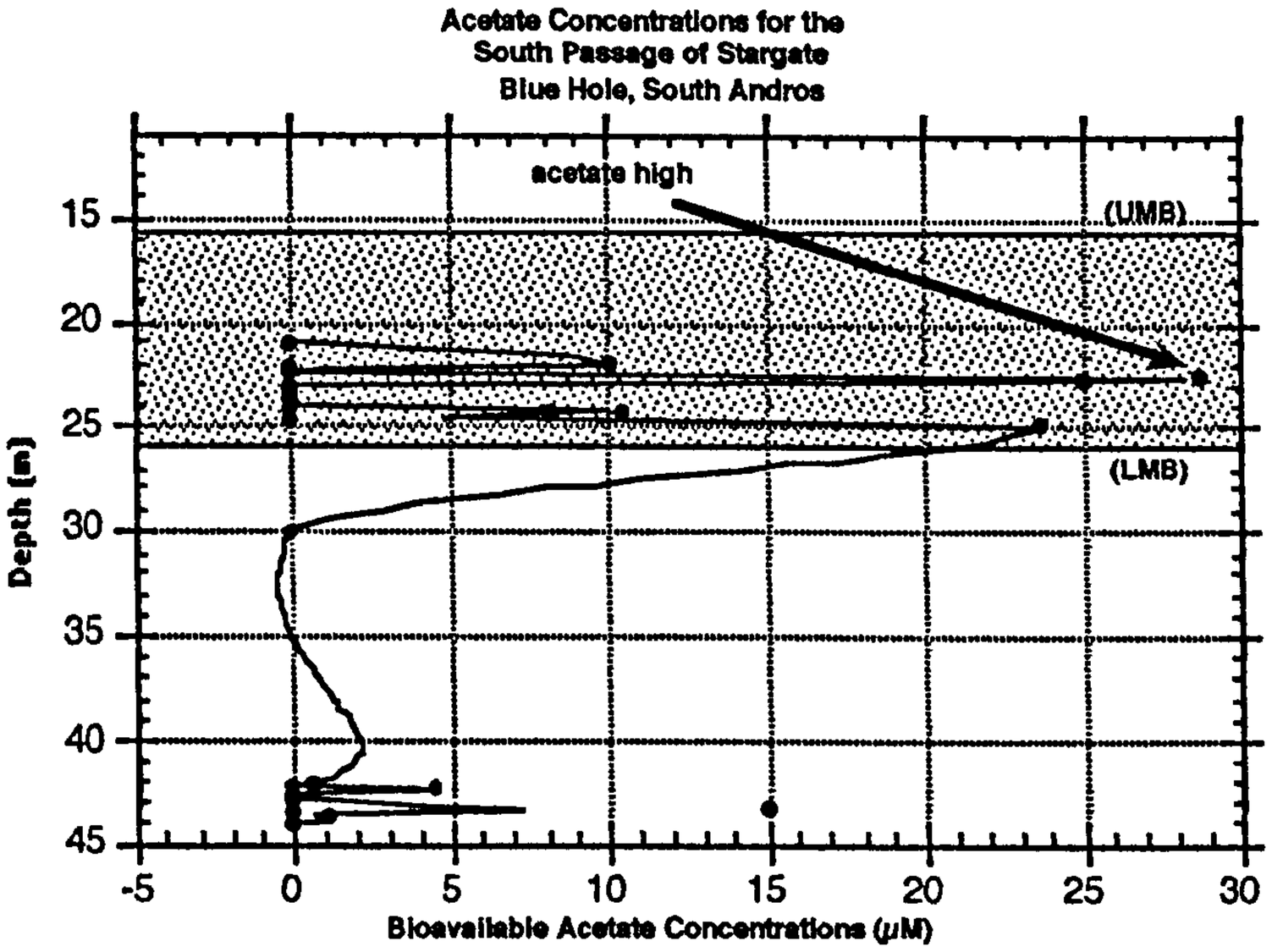


Figure 5.33: Background acetate measurements from samples collected in the south passage in Stargate Blue Hole. The dot marked as acetate high can be in the (POC) graph (Figure 5.32) and the DOC graph (Figure 5.31).

In quite a few of the Stargate samples acetate concentrations were below detection, a situation which occurred in some samples from all other sites on Grand Bahama.

5:6 Bacterial Activity

5:6:1 ^{14}C Acetate Turnover

Of the 2 and the 6 hours incubation rates, the 2 hour incubations, in most samples, had the highest turnover results. Three depths of peak activity for the 2 hours incubation times within the water column occur at 22.6 m with a measurement of 0.06 μM/hr, 24.8 m, and 0.098 μM/hr. The largest peak is at 42.4 m with a measurement of 0.31 μM/hr.. Broadly similar results were obtained with the 6 hour incubations, a larger peak occurs at 22.6 m, 0.09 μM/hr., a small one at 24.8 m, 0.02 μM/hr and the smallest at 43 m 0.056 μM/hr.

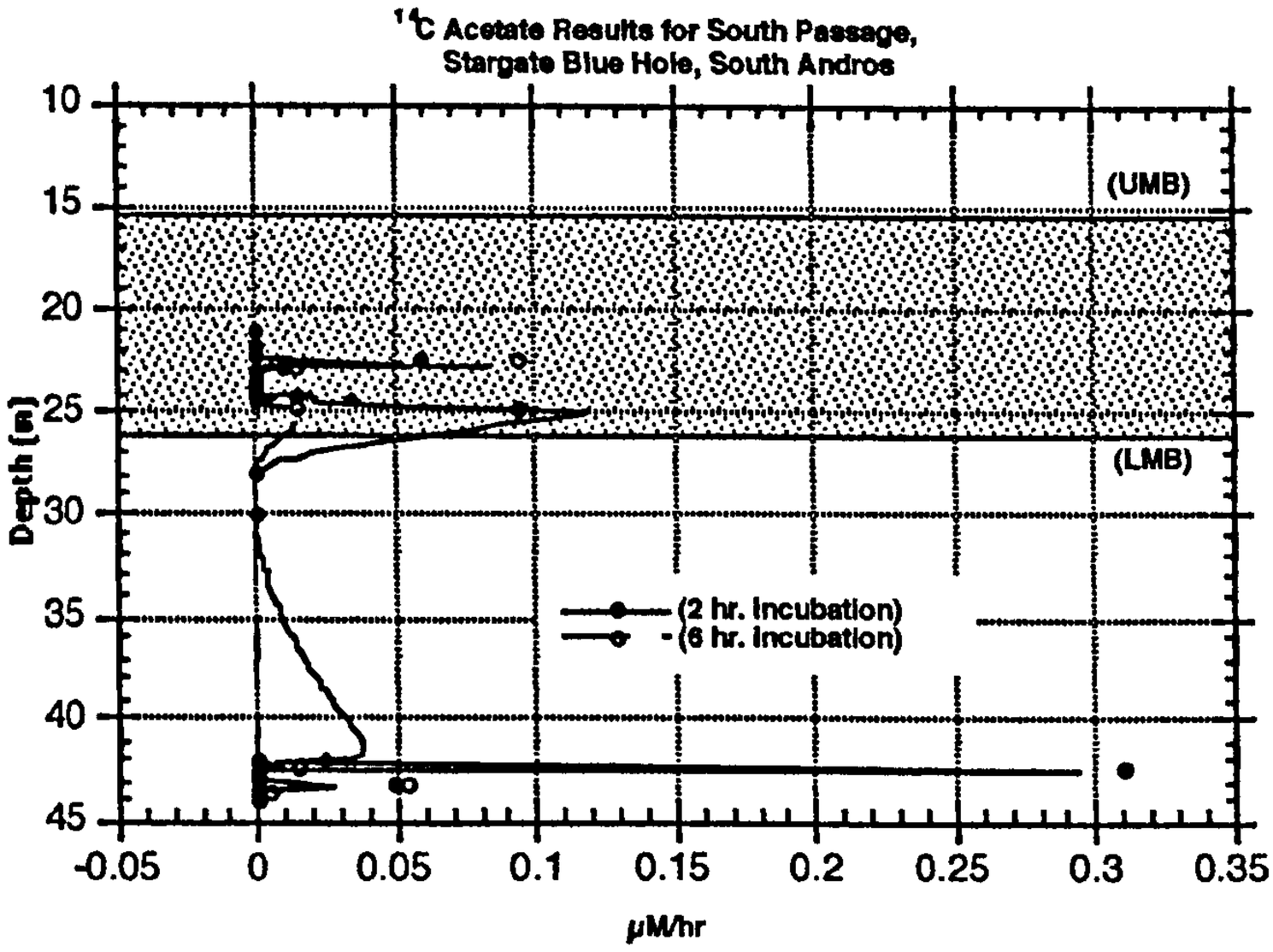


Figure 5.34: ^{14}C Acetate results for samples collected from the south passage in Stargate Blue Hole

Both areas in the water column which generate peak results are in close association with areas where major changes occurred in DO (refer to figure 5.30) values. The first peak activity comes just prior to a small oxygen peak at around 26 m, just above the LMB, and the other peak at depth occurs just prior to DO values dropping below 1 mg/l. Looking at the DOC graph (Figure 5.31),the highest DOC values were found at 24 m and 43 m. Bacterial counts are also the highest at 24 m (Figure 5.40) and 43 m.

5:6:2 ^{14}C Bicarbonate Utilization

Of the 2-day and the 5-day incubation rates, again, as observed in the acetate results, the 2-day rates were higher. The highest rate occurred at 22 m with a rate of 6.8 μM^{-1}/day. The second highest rate came at 23 m with a result of 4.3 μM^{-1}/day, followed by 24.8 m, 4.2 μM^{-1}/day and 42 m, 1.2 μM^{-1}/day.

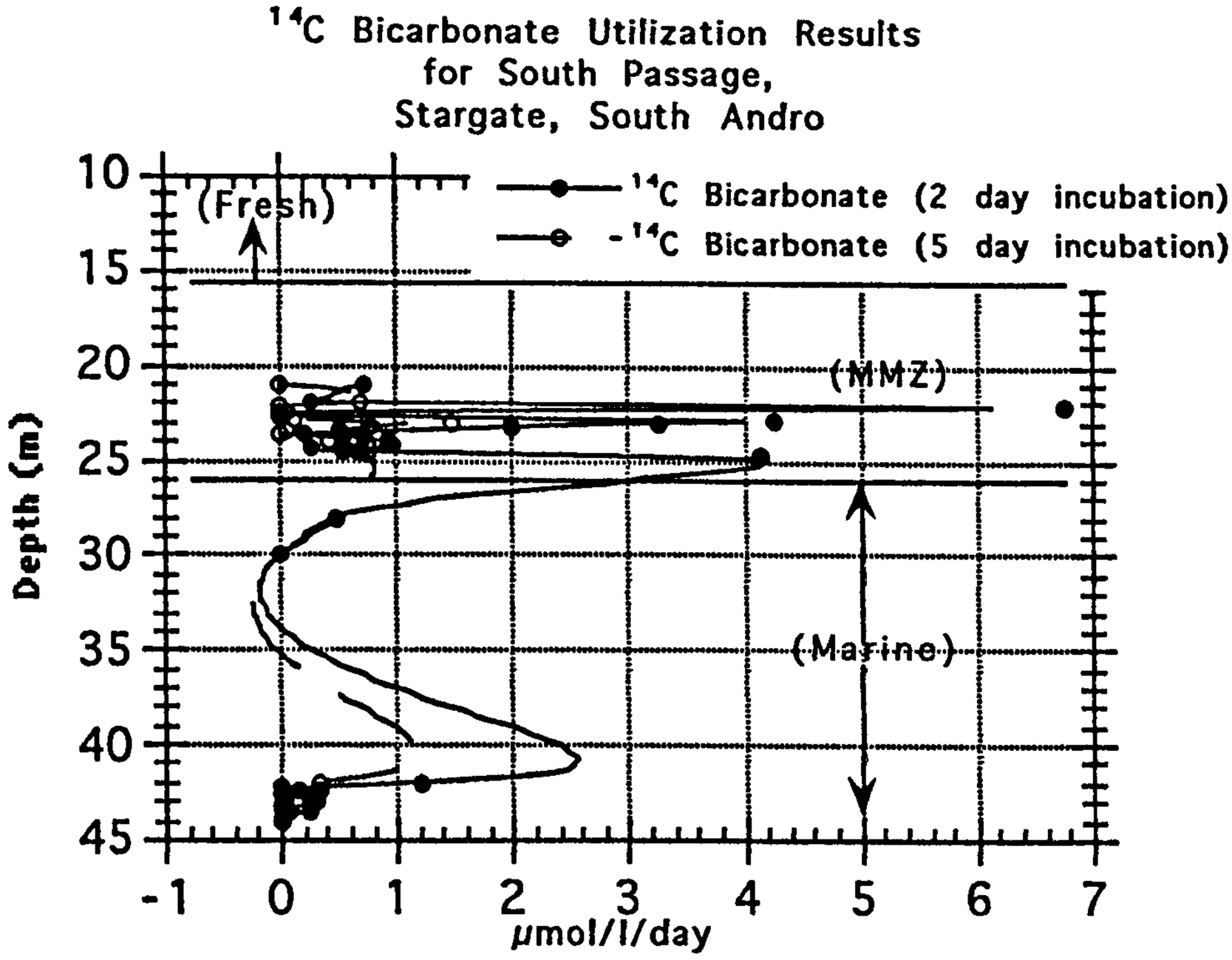


Figure 5.35: ^{14}C Bicarbonate measurements made samples collected from the south passage in Stargate Blue Hole

Activity within the water column appears to correlate within bacterial numbers, DOC, and POM.

5:6:3 Sulphate Reduction

Samples for ^{35}S estimated turnover rates were filtered and non-filtered. The 1996 sulphate samples were filtered to remove POM where is was suspected the sulphate reduction was still taking place. It was possible that bacteria that were attached to particles were creating microaerophilic or anoxic environment within particles. To test this possibility, sulphate reduction measurements were taken from filtered and non-filtered samples.

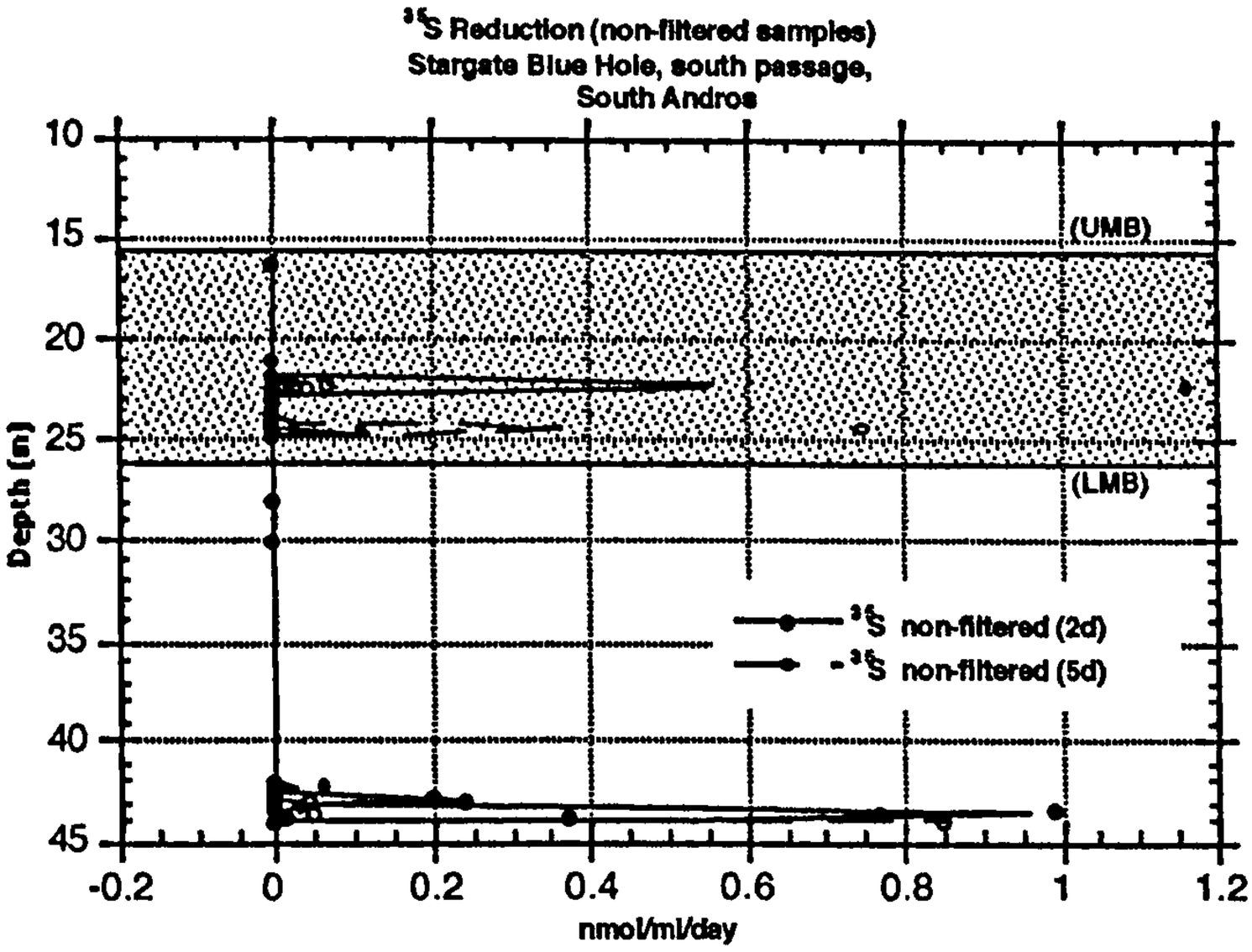


Figure 5.36: Non-filtered samples for ^{35}S reduction results for samples collected from the south passage in Stargate Blue Hole

The results however, (Figure 5.36 and 5.37) were as expected. It appears that rates are slightly higher in the filtered samples than in the non-filtered. The 2-day rates reduction rates again are higher than the 5-day

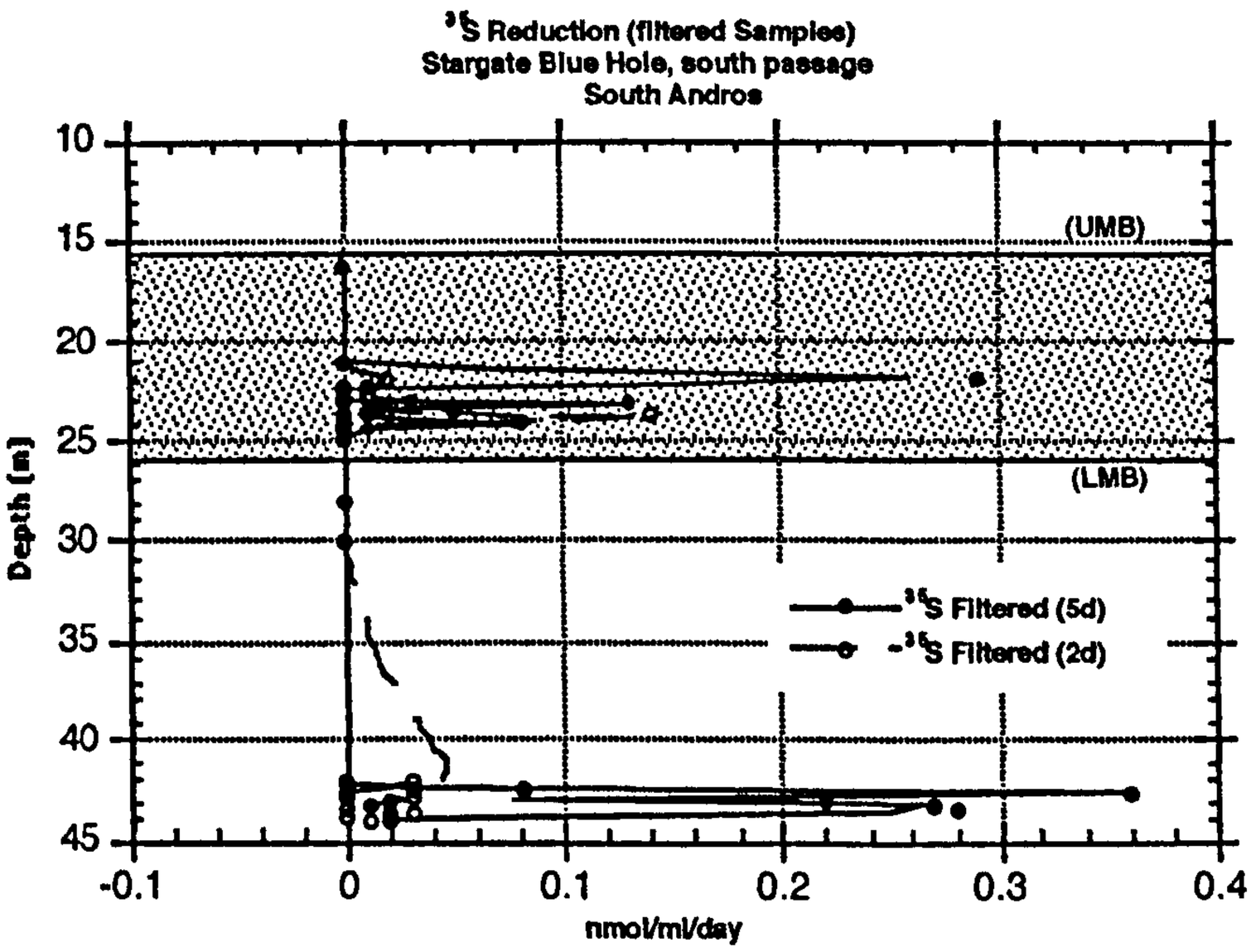


Figure 5.37: Filtered water samples for ^{35}S reduction rates for samples collected in the south passage in Stargate Blue Hole. Note the similar peak at 22 m which is found in figure 5.31; 5.32; 5.33; 5.34; and 5.35.

incubation rates in both, the filtered and the non-filtered samples. Explanations will be given in the discussion. Although the rates are not high, they may be significant for these environments. Maximum turnover rates for both the filtered and non-filtered samples occur between 42.8 m and 43 m. The 5-day incubation rates for all samples (Figure 5.36) were all negative whereas 5-day rates in the filtered sample were mostly positive, especially from samples collected at depths between 22 m and 24 m and 42 m and 44 m.

Again, as seen in the Lucayan and Owl's Hole results, sulphate reduction is occurring in waters with low levels of DO, although maximum measured activity occurs in waters very close to being anoxic.

5:6:4 Bacterial Growth Rates

Thymidine incorporation results from samples collected within the south passage in the Stargate blue hole showed that the bacteria are indeed growing. Two areas within the water column specifically showed heightened activity. These were samples from 24.2 m and 44 m (Figure 5.38). Cell production rates occurred at 44 m with rates of 861,000 cells/ml/day. The second highest rate for cell

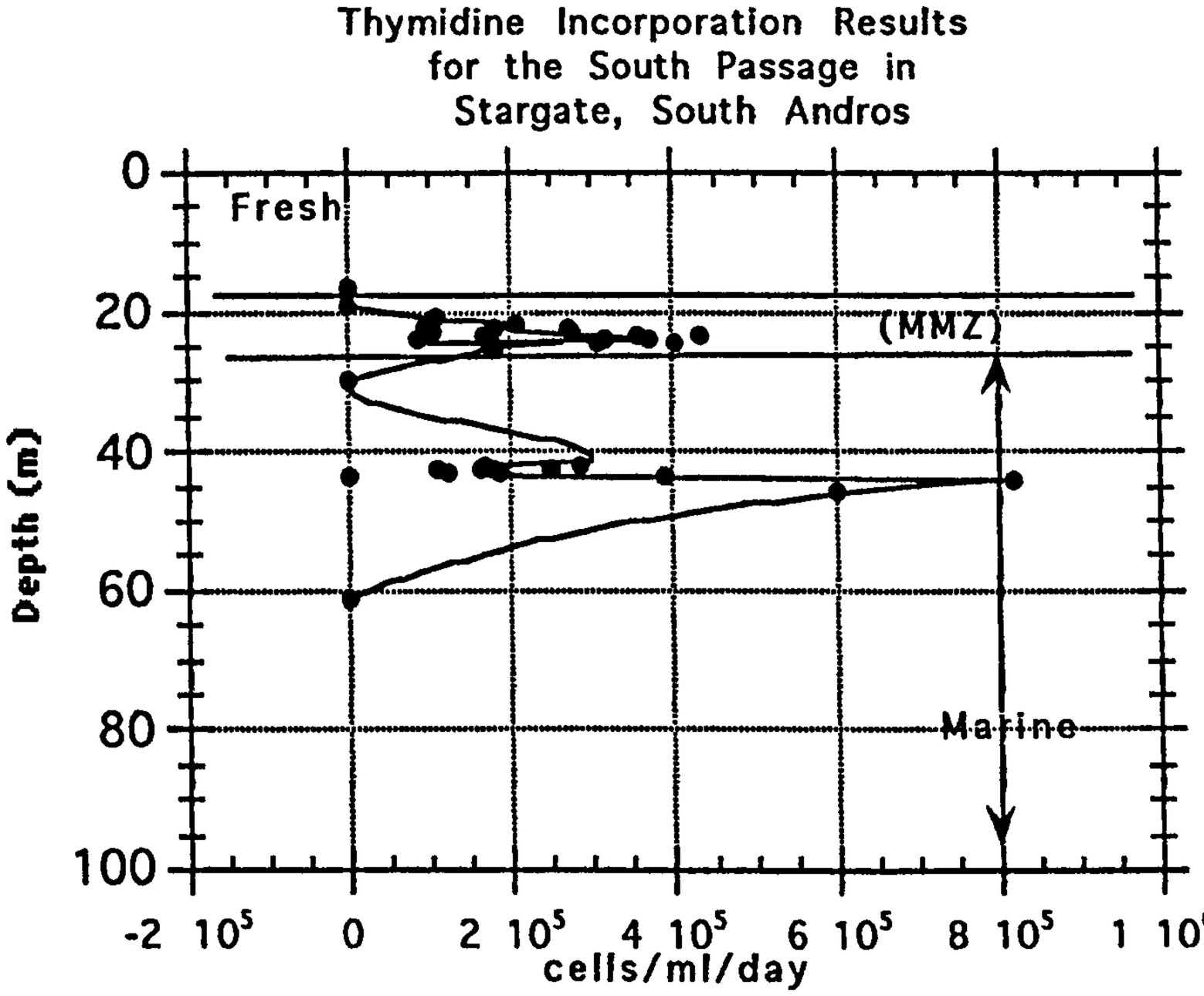


Figure 5.38: ^{3}H **Thymidine incorporation results for samples collected in the south passage in Stargate Blue Hole**

production occurred at 24.2 m with a rate of 440,000.00 cells/ml/day. An additional find at the 43 m mark was a substantial population of a new species of copepod yet to be named (personal communication, Audun Fosshagen). What makes this species of copepod so unusual, besides the enormous numbers of them within this layer of water, was that this species had only soft and extremely small appendages, indicating that its food source had to be

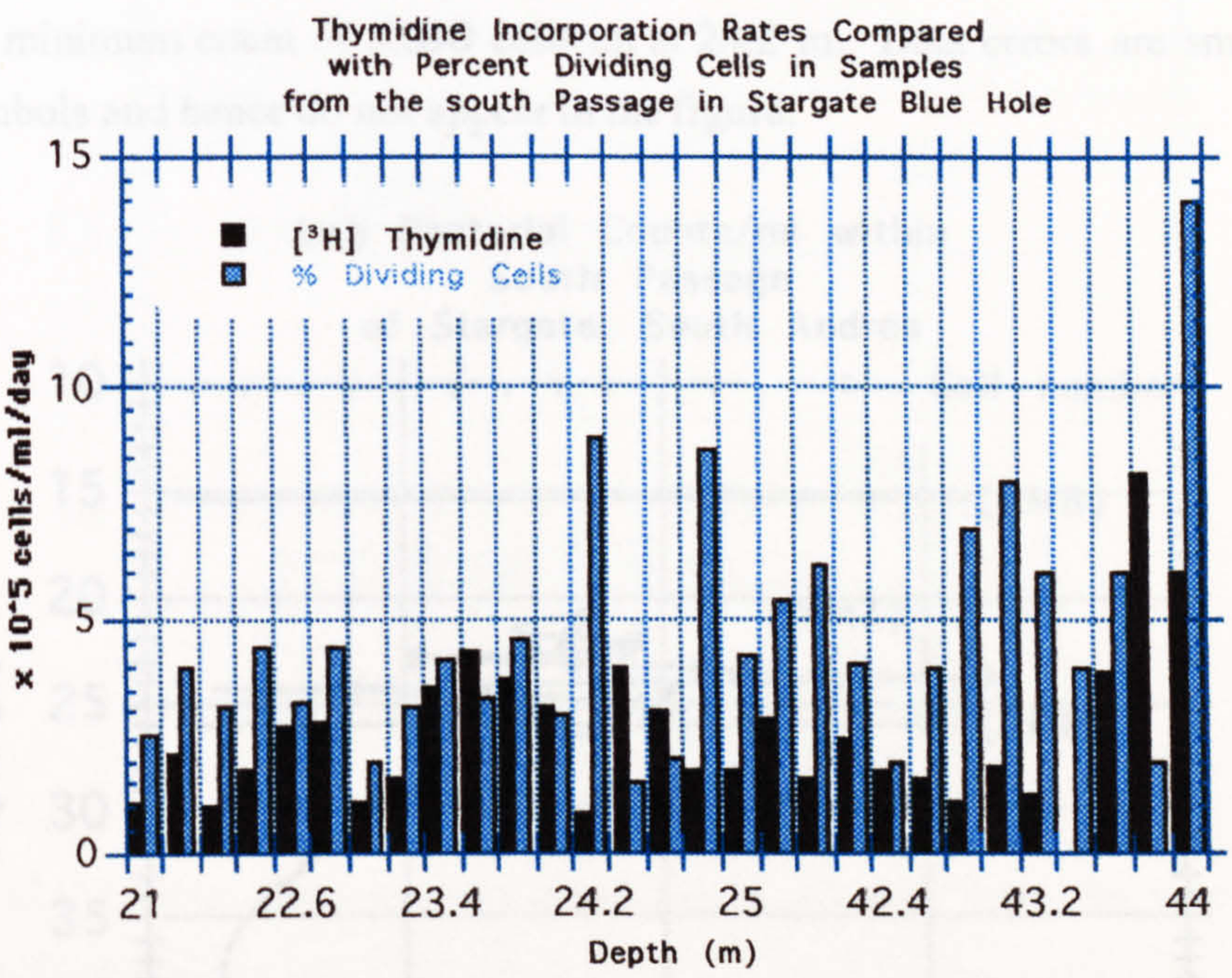


Figure 5.39: [³H] Thymidine results compared to percent dividing cells in the south passage in Stargate blue hole

equally soft and small. This may have significant bearing when trying to describe the dynamics of this layer of the water column.

Statistical correlation proved to be negative overall and within individual water bodies when comparing thymidine results and percent cell division. Obviously, thymidine incorporation into DNA and no cell division would bring into question the results. Three samples, at 24.4 m, 24.6 m and 43.8 m have a lower percentage of dividing cells than thymidine incorporation rates. All other sample results show cell division to be greater or better than 50% of the thymidine rate.

5:7 Bacterial Counts

5:7:1 Total Bacterial Counts

All samples collected contained bacterial cells (Figure 5.40). Most of the bacteria seem to be concentrated at the base of the MMZ and near the microaerophilic zone in the 40 m depth range. Bacterial populations ranged from the highest count of 130,436 cells/ml (n=3) to a minimum count of 3,890 cells/ml at 24.2 m. Data errors are smaller than plotted symbols and hence do not appear in the figure.

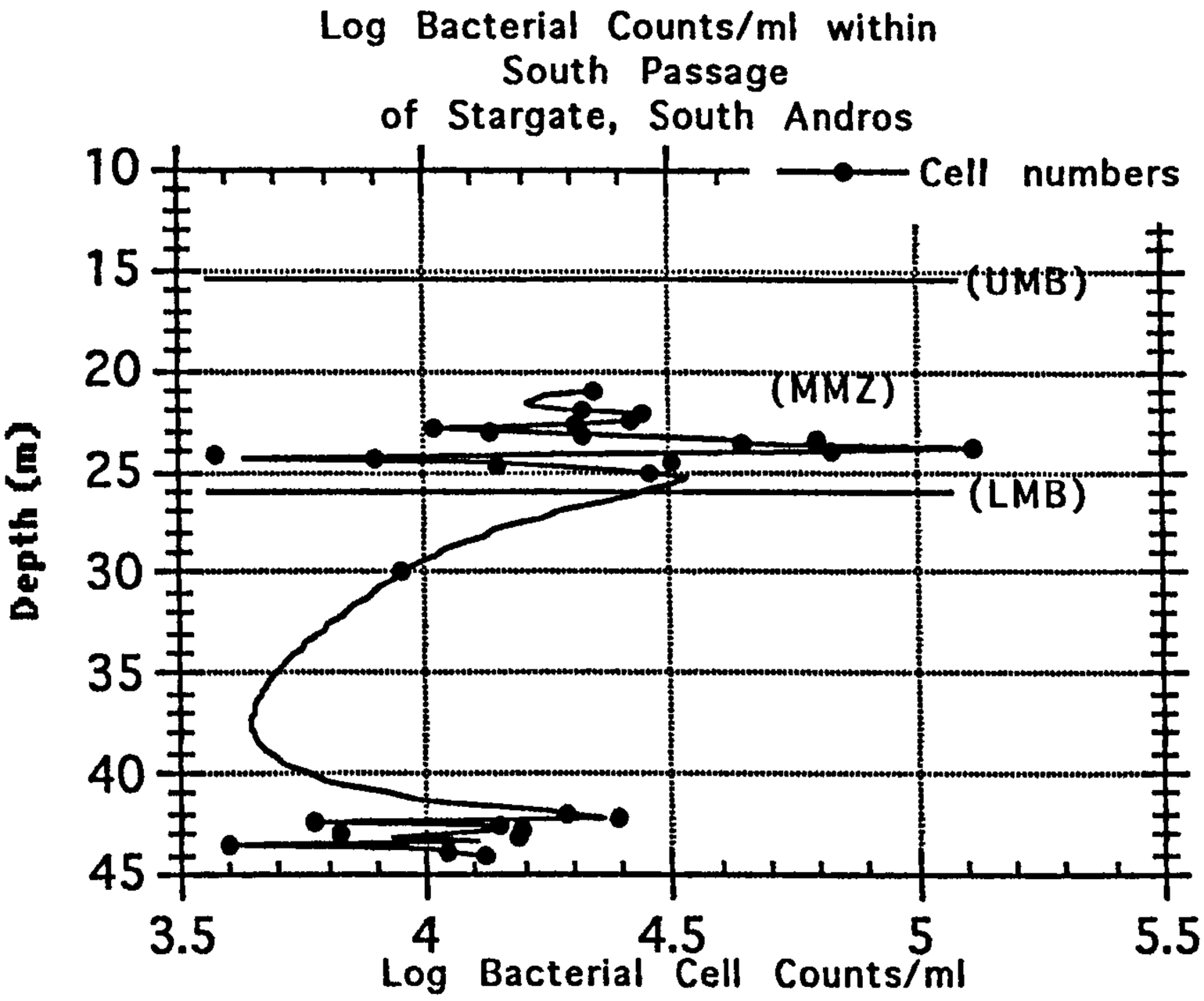


Figure 5.40: Total bacterial counts for samples collected in the south passage in Stargate Blue Hole

While counting bacteria in these samples, it was noticed that cell numbers could increase or decrease dramatically within 20 cm of depths. This suggests that bacteria found in layers within the water column may be responsible for the fine banding that divers observe. This suggestion is supported by the fact that copepods are also found in layers.

5:7:2 Dividing Cells

All samples collected from the south passage of the Stargate blue hole contained dividing bacterial cells (Figure 5.41). The reliability of this data has been supported by

the thymidine results already seen in Figures 5.38 and 5.39. Areas of the water column with the highest cell dividing activity was the area just above the LMB within the MMZ and the area between 42 m and 46 m. At 43.4 m, 14% of

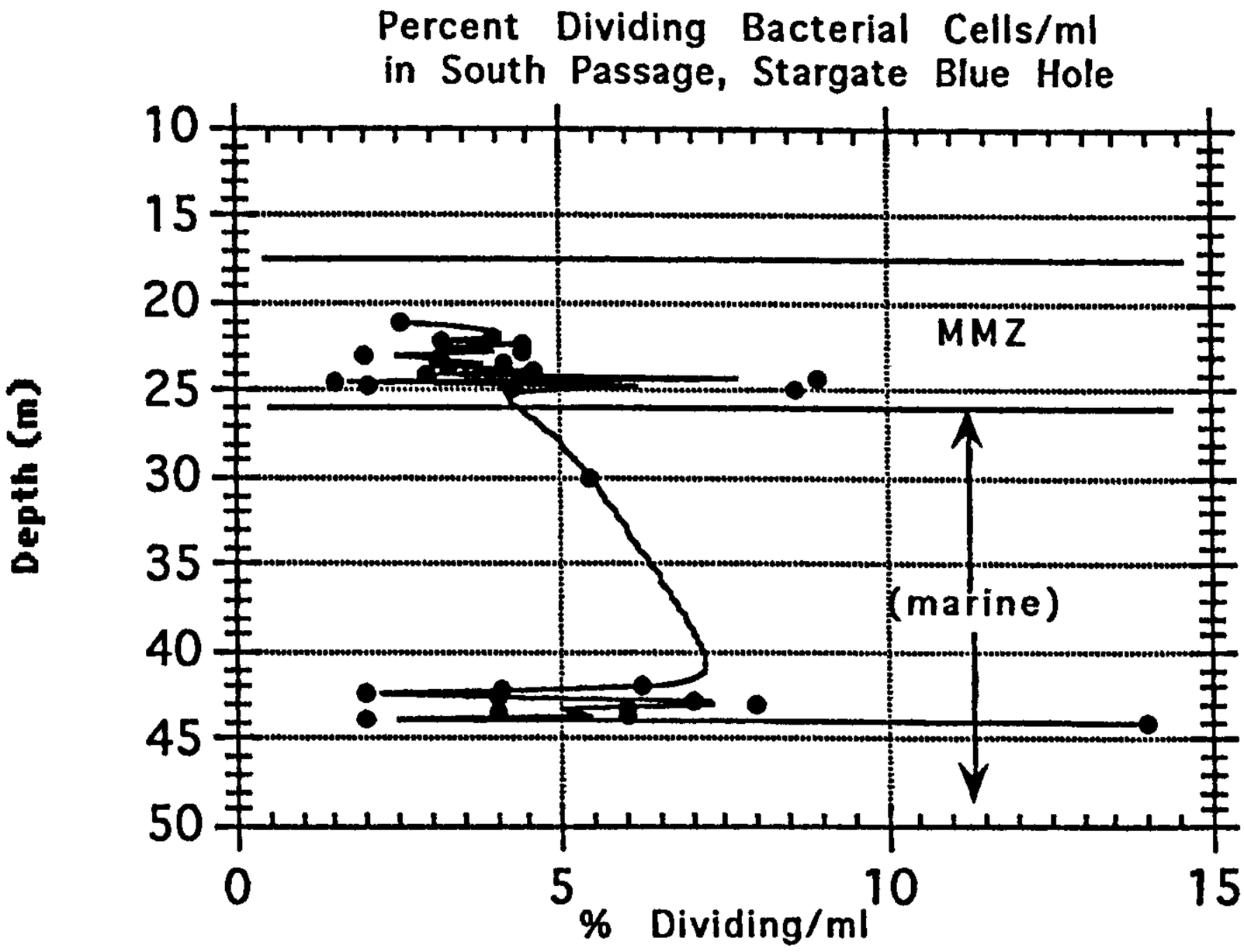


Figure 5.41: Percent dividing cells measured in samples from the south passage in Stargate Blue Hole

the total cells were dividing. Two other samples showed 12% of the cells to be dividing at 42.8 m and 43 m. It appears that deep water samples have the higher percentage of dividing cells than the more shallow water samples. The overall average for samples collected below the LMB was 6.9% whereas the average within the MMZ was 4.2%.

5:7:3 Bacterial Counts on Particles

Just below the UMB, bacteria counts associated with particles within samples collected in the south passage of the Stargate blue hole are the highest (Figure 5.42) with a value of 185 cells/ml at 15.5 m. With depth however, the values decrease except for a few areas above the LMB where values increase on and off over a vertical distance of 21 m to 25 m.

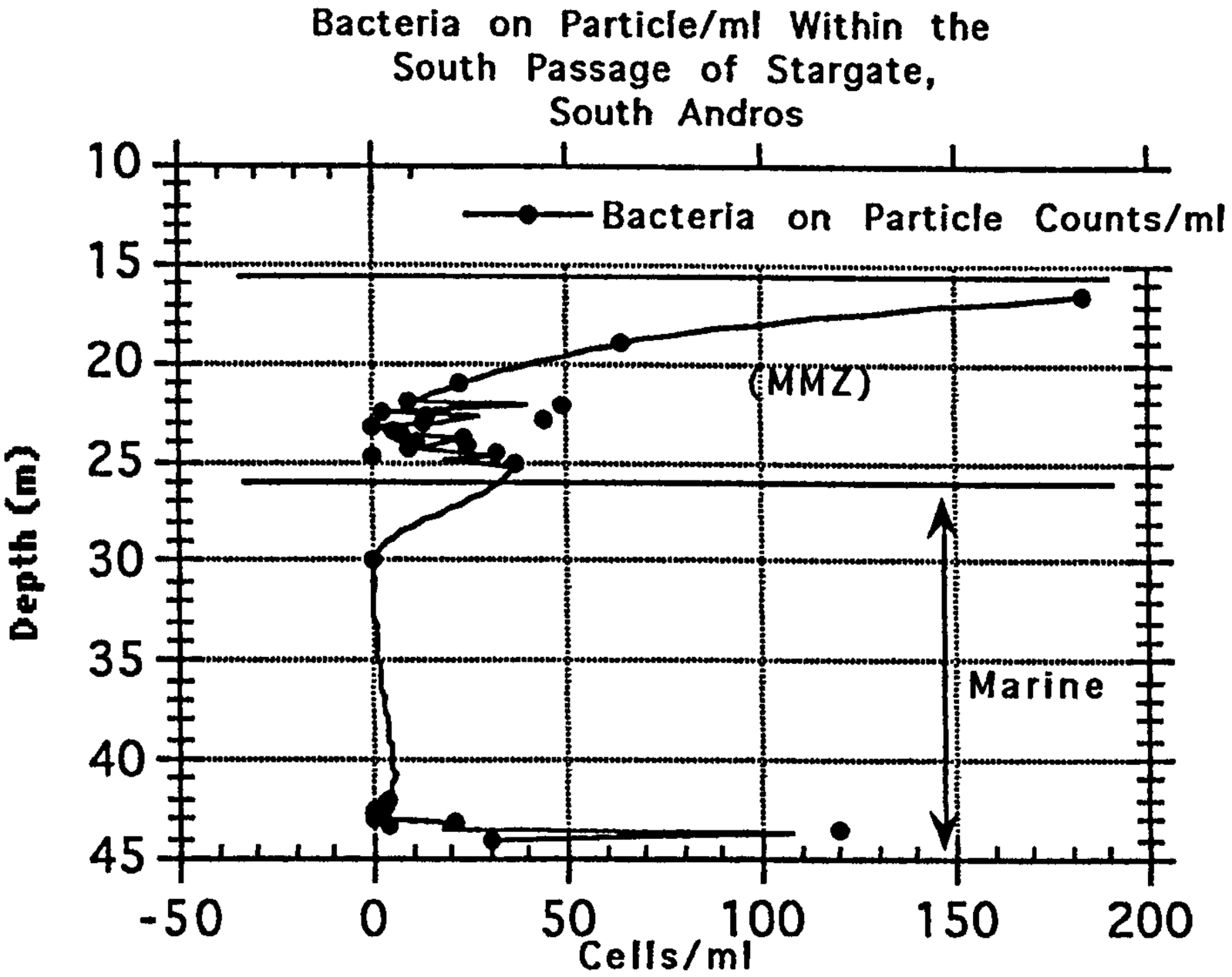


Figure 5.42: Bacteria-on-particle counts for samples from the south passage in Stargate Blue Hole

Below the LMB, values remain very low, in some cases, "0", to the depth of 42.8 m. Here the value increases slightly except for the sample collected at 43.8 m which gives the second highest value of all collected samples. At this depth the population was 120 counts/ml. All counts are accurate with zero cell counts in the blanks (n=3) already subtracted. In figure 5.43, POM was plotted against bacteria-on-particle for comparison.

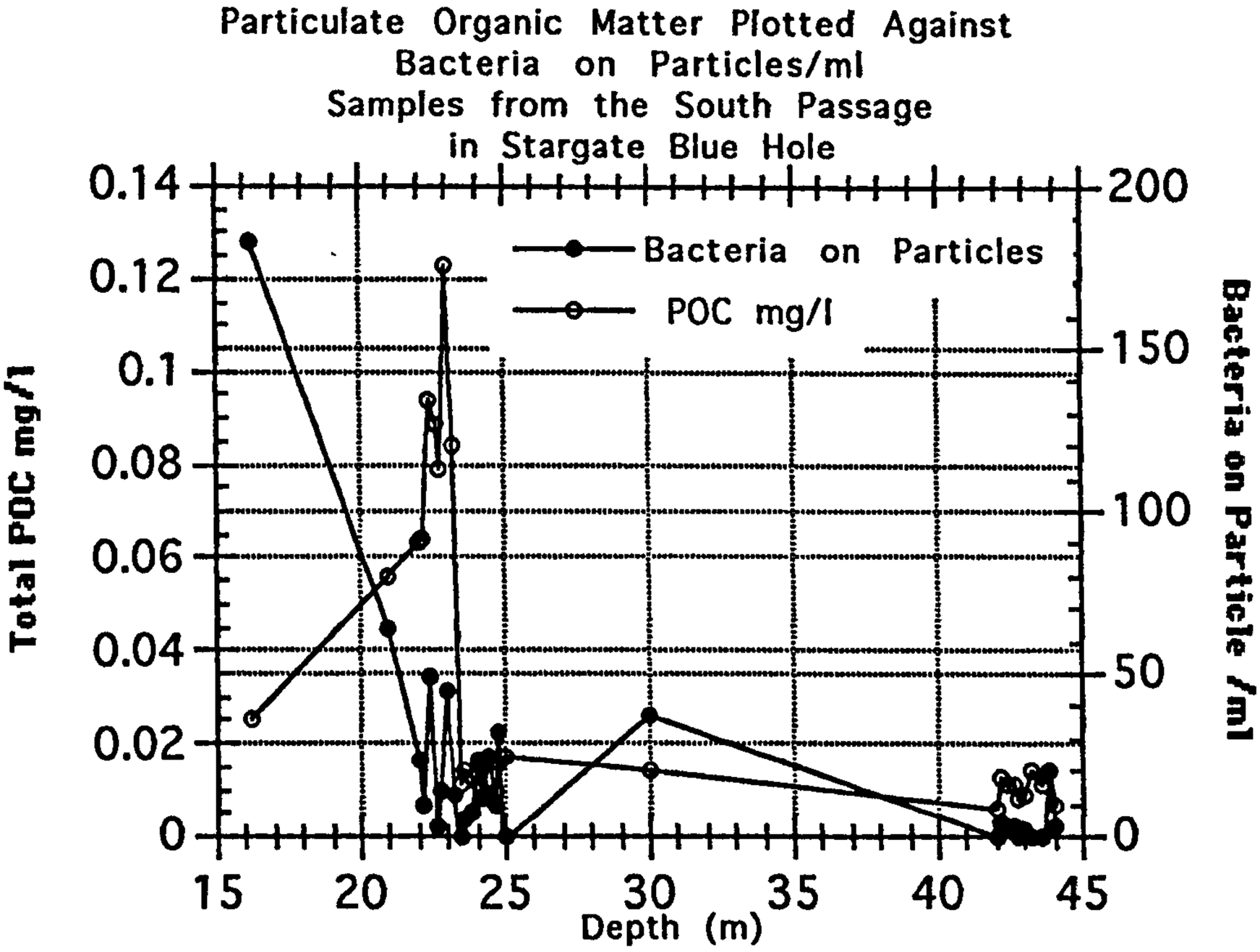


Figure 5.43: **Bacteria on-particle-counts plotted against particulate organic matter found within samples collected from the south passage in Stargate blue hole.**

Except for the first sample there appears to be only a small correlation between POC and bacteria-on-particle. This would actually support the ^{35}S filtered reduction rates (Figure 5.37). Some POC samples register "0" where bacteria have been counted as being on particle. This may be explained by the fact that particulate organic material is not evenly distributed within water strata. It is like seaweed floating on the oceans surface. A surface sample may be collected with some seaweed and it is equally possible to collect a surface sample without seaweed. This same analogy applies to sampling within these cave sites.

5:7:4 Bacterial Counts off Particles

Bacteria-off-particle counts (Figure 5.44) are significantly higher than bacteria-on-particle (refer to Figure 5.43). This, however, is not unexpected because of the clarity of the water in the Stargate blue hole. Within the vertical water column, the highest number of bacteria-off-particle is at the depth of 23.8 m, with a count of 582 cells/ml. Another peak was at 22.4 m with a count of 420 cells/ml. A slightly elevated cell count was found at

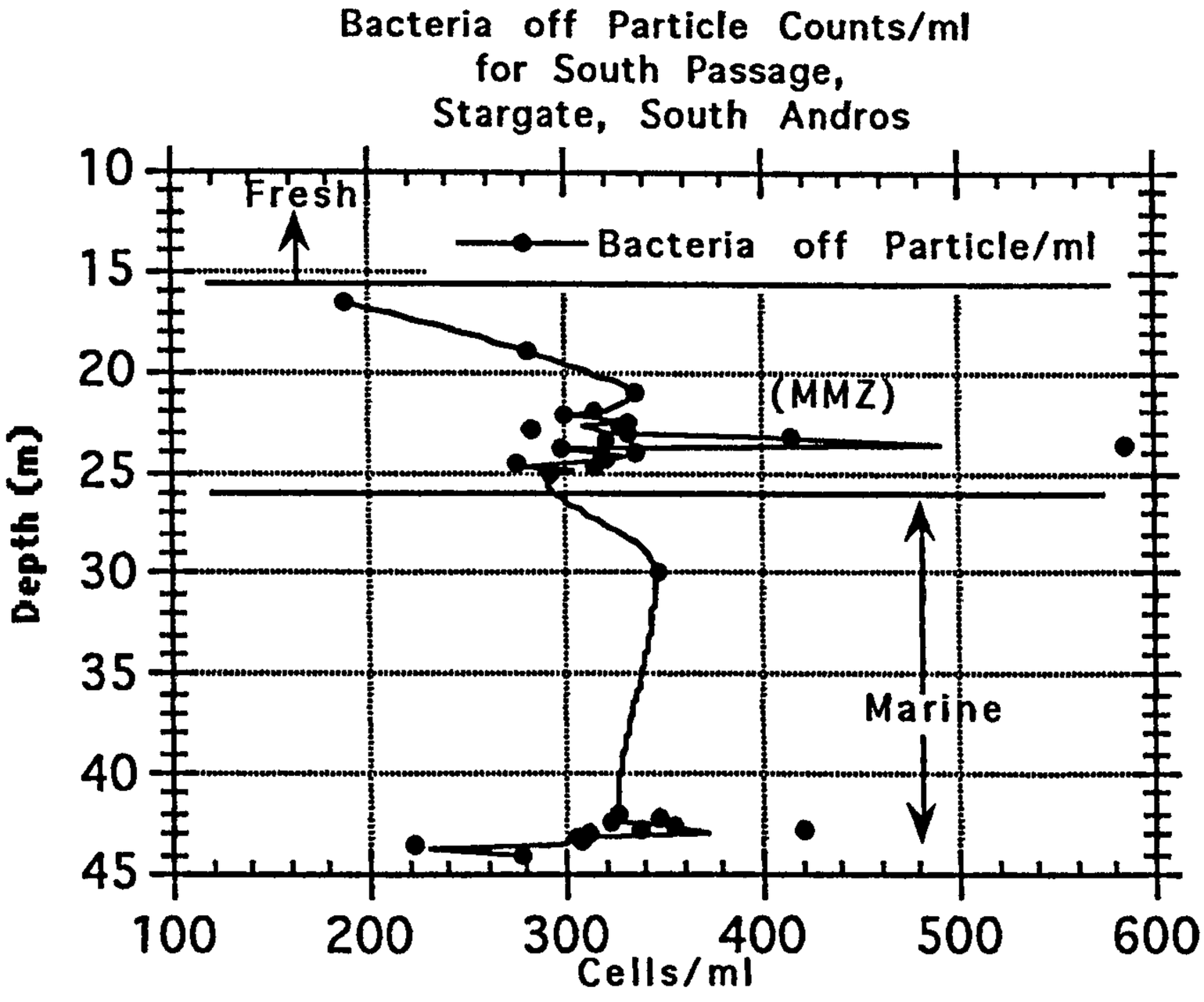


Figure 5.44: Bacterial-off-particle counts for samples from the south passage in Stargate Blue Hole

42.8 m with counts of 420 cells/ml. All samples were positive for bacterial cells and, except for the three elevated peaks, overall cell numbers were evenly distributed throughout the vertical water column with an average of 342 cells/ml within the MMZ and 306 cells/ml from below the LMB to the maximum sampled depth.

5:8 Geology

5:8:1 Thin Section Petrology

Hand samples collected from Stargate during the Andros 1987 Expedition were given to Mr J. M. Dawans of Shell KSEPL, Holland, for thin-sectioning. All samples from this site were highly altered and the composite grains virtually all but dissolved away except for micrite envelopes (Table 5.1), giving a ghost view of what the original composite grains were. Microcrystalline cement was the main type of cement found within these samples and a mixture of bioclasts and ooids (refer to Table 1.1 and 5.2) appear to be what the original composite grains were. Both bioclasts and ooids are originally composed of aragonite, a metastable form of calcite. This finding is important for explaining rates of dissolution. Some composite grains will

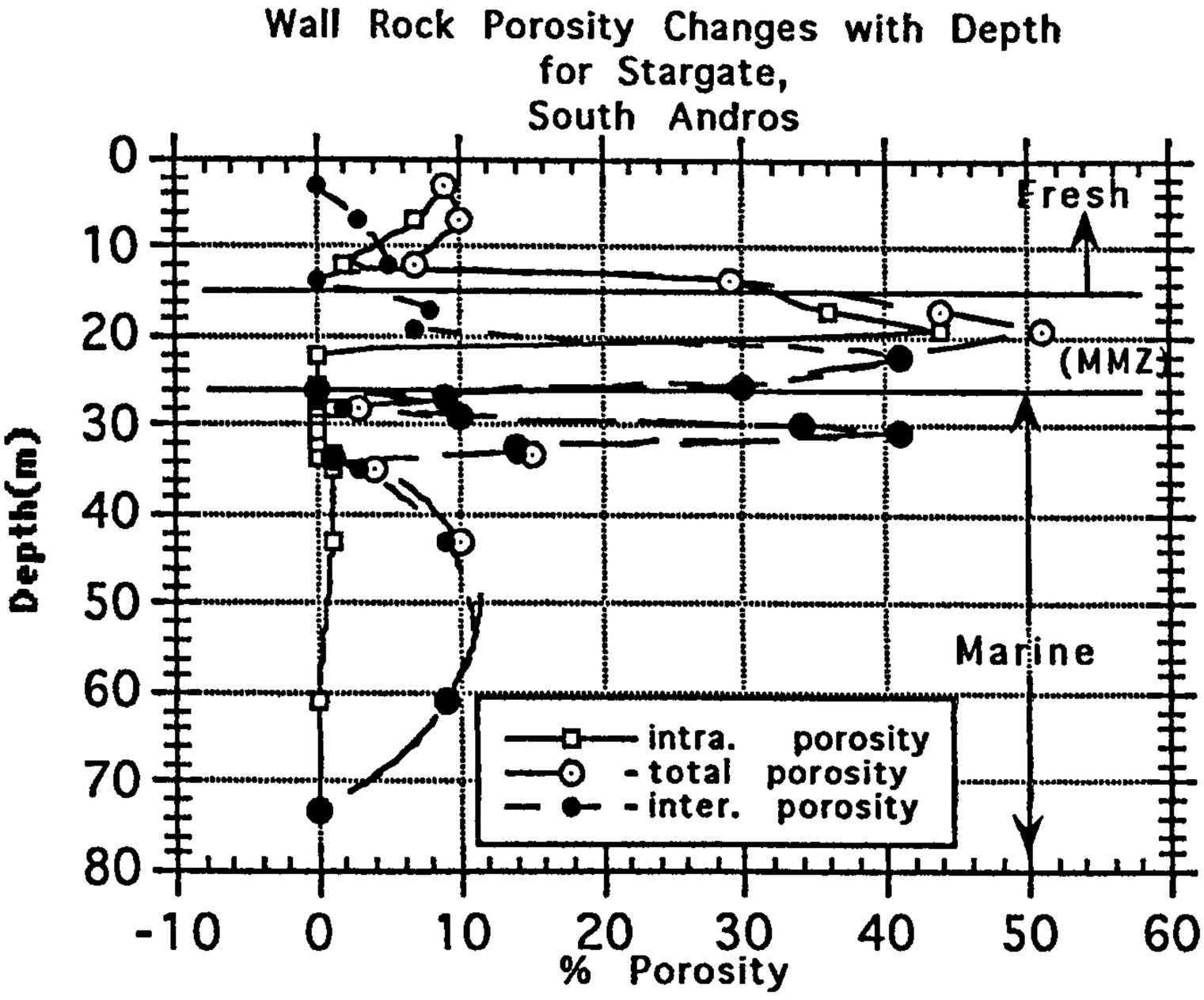


Figure 5.45 **Profile with depth showing percent porosity as (inter, intra and total).**

dissolve more readily based on chemical makeup and shape, whereas other grains will be more resistant to dissolution for the same reasons (Folk, 1974, Ehlich, 1990). Of great interest to this project is the amount of porosity within the wall rock of the caves and at what depth and associated with what body of water the highest or lowest percentage of porosity occurs. In Stargate, maximum porosity was found within the MMZ boundaries, with intraparticle (Table 5.1) being the most dominant form of porosity. Within the fresh water body, intraparticle porosity dominates again, however below the LMB, interparticle porosity (Table 5.1) is the most prevalent form.

Table: 5.1. Petrological definitions of descriptive terms used in rock analysis

bioclast	material derived from the supporting or protective structures of animals or plants
interparticle porosity	the porosity **between** particles in a rock
intraparticle porosity	the porosity **within** individual particles of a rock
micrite	chemically precipitated carbonate mud with crystals less than 4 microns in diametre
micrite envelope	a thin coating of micrite around allochems, particularly skeletal grains. It is produced by coating or boring algae, or mechanical adhesion of carbonate mud.

6

Discussion and Conclusions

Introduction

The objective of this study was to describe the geochemical and physical parameters within blue holes environments, to determine the distribution of bacteria by direct counting methods, to quantify bacterial activity using radiolabeled carbon and sulphate tracers, to determine bacterial growth rates using [^{3}H] methyl thymidine incorporation into bacterial DNA, assess a potential for cave development by bacteria through alteration of water chemistry and to identify their presence within wall rock material. Field studies on Grand Bahama and South Andros provided insight into how complex and dynamic the blue hole environments are within modern carbonate platforms.

In this final chapter I will review data concerning these objectives, followed by a comparison and discussion of differences between the varied cave systems, i.e., lens base versus fracture system and how the genesis and location of a cave system may dictate the geochemistry and ecology of these submerged environments.

6.1 Geochemistry

Geochemical data, salinity (Figure 4.16 A, 4.42 A, and 5.19), dissolved oxygen (Figure 4.16 C, 4.42 C, and 5.30), pH (Figure 4.16 D, 4.42 D, and 5.25), acetate (Figure 4.18, 4.44, and 5.33) alkalinity (Figure 4.12, 4.39, and 5.27) DOC, (Figure 4.17, 4.43, and 5.31) and temperature (Figure 4.16 B, 4.42 B, and 5.21, 5.22) all show that each cave and site within the same cave system is vertically structured (Table 6.1) with steep chemical gradients and interfaces: environments where bacterial processes would be anticipated to be concentrated.

6:1:1 Salinity

Salinity gradients at all three sample sites demonstrated the existence of three separate bodies of water; a fresh or brackish lens, a mixing zone and a marine or saline zone

(Figure LC 4.16 A, OH 4.42 A and SG 5.19). Salinity between the three sites ranged between 0.4 g/l to 36.5 g/l (Figure LC 4.16 A, OH 4.42 A and SG 5.19). The bottom section of the water column at all three sample sites was marine. This is the case for all caves explored to date in the Bahamas.

The quality of the fresh water and the thickness of the lens varied enormously between all study sites. Referring again to the USGS standard for drinkable freshwater, water ≥ 0.5 g/l salinity mentioned in earlier chapters, Lucayan Cavern and Owl's Hole were the only sites containing drinkable water. Stargate on South Andros according to these standards had no fresh water at the time of sampling. After a major storm, such as a hurricane, the fresh water lens can be several metres thick and could be drinkable however it is still unsuitable for drinking because the water is very laden with tannin. Stargate to date is the only system observed to have occasionally a fresh water lens. Lucayan Caverns and Owl's Hole on the other hand always have a fresh water lens and its thickness has not been seen to vary over a 12 year data history.

Mixing zone thickness' varied within and at all three cave sites. This is of particular interest because variation in its thickness has not been addressed in the Ghyben-Herzberg Model (Figure 6.1) but also the depiction of the fresh water lens boundaries being the shape of a perfect lens is known to be unrealistic. Although the Dupuit Ghyben-Herzberg takes into account the topographical morphology, size and shape of the island which will to some degree influence the shape and location of the fresh water lens, no where does it take into account the affect of lateral tidal water pressure squeezing or changing the shape of the lens or variations seen within the mixing zone which would also potentially cause variation in the shape of the lens. This is one of the many problems of trying to introduce models of environments where very little information is available and the environment is not truly understood. Unfortunately, very few people challenge these models and as a result, environmental decisions are based on these models with potentially disastrous results.

Interest in the variation in the shape of the fresh water lens was brought to the attention of this study after mapping of the Owl's Hole Mermaid's Lair system (Figure 6.1). Mermaid's Lair is the name of another opening to the same Owl's Hole cave system (Appendix 1). Naming of the two entrances occurred before it was known that the cave system connected underground. The entrance known as Mermaid's Lair is 120 m in from the coastline and the Owl's Hole openings is 965 m north from the Mermaid's Lair entrance.

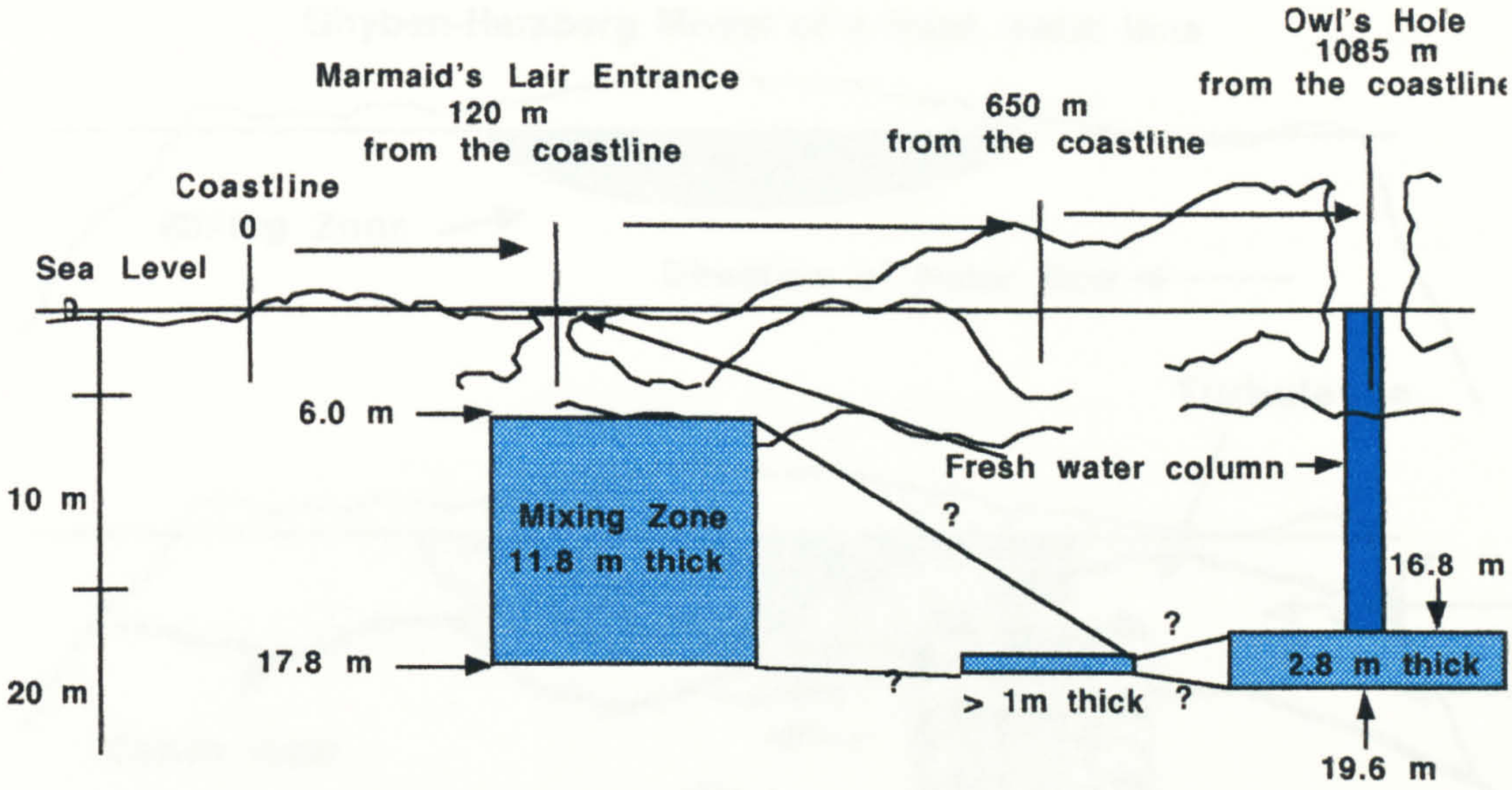


Figure 6.1: Changes in mixing zone thickness seen with increased distance from the shoreline. Mermaid's Lair, the second entrance to Owl's Hole, connects underground to Owl's Hole making it one cave system with two openings 965 m apart in measured surface distance.

As it can be seen, at the Mermaid's Lair entrance, the mixing zone is 11.8 m thick and thins to less than 1 m thick, 650 m running north underground. However in the Owl's Hole entrance, 965 m north of the Mermaid's, the mixing zone thickens again to 2.8 m. Reasons for this may be 1) varying porosity of the wall rock causing variation in speed of water flow, 2) distance of the fresh water lens from the coastline, and 3) a combination of both 1 and 2 (Figure 6.2).

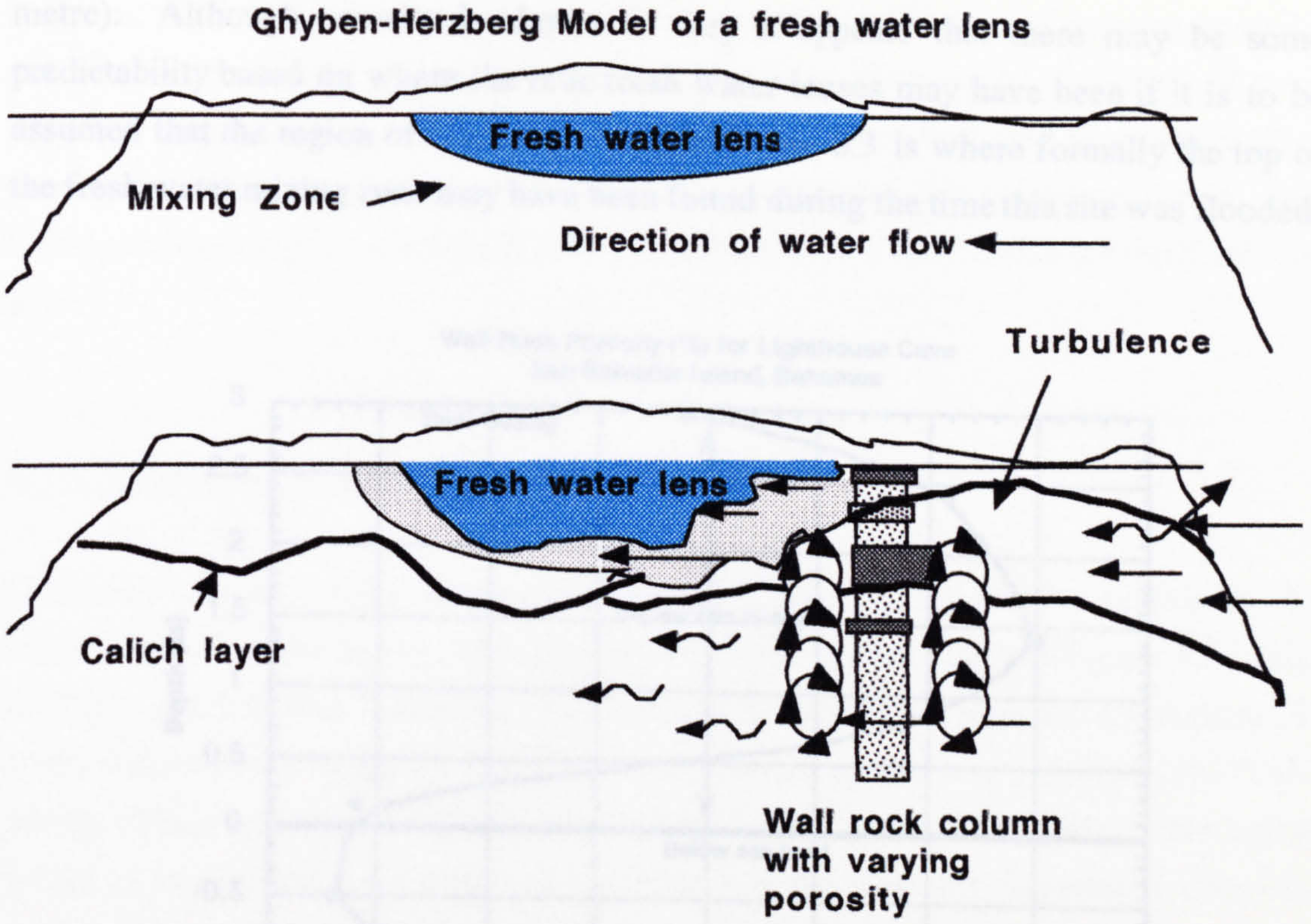


Figure 6.2: Proposed configuration of the fresh water lens and mixing zone.

Wall rock porosity variation can be seen in caves which are currently dry and above sea level (Figure 6.3) and where sections are below sea level (Figure 5.15 and Figure 6.4). The caves found currently 6 metres above sea level though out the Bahamas are referred to in literature as "Flank Margin Caves" (Mylroie and Carew, 1990) suggesting that the caves formed at the flank margins of the ridges. Based however on the many blue holes which have been explored to date, the so called "Flank Margin Caves" are very young (in reference to development) relic blue hole caves. These are caves which do not have a long submergence history, ~15,000 years (Mylroie and Carew, 1990) and therefore are not as large as the lens based caves we dive today. The dry caves also do not have the repeated submergence history of the currently flooded blue hole cave systems. For the purpose of this section of the thesis, these caves will be referred to as relic blue hole caves or just relic caves. These same relic caves are known to exist below actively forming caves as well.

What interesting to observe between the dry relic caves and the blue holes is the similarity of porosity changes (Schwabe et al., 1993) within the wall rock over similar distance (Figure 6.3 and 6.4). In Lucayan Caverns (Figure 6.4) over a 1 m distance from shallow to deeper water, porosity increases by 13%. From the top of the mixing zone to the bottom, a distance of 2 m, porosity increases by 23% (averaging 11.5% per metre). In Lighthouse Cave (Figure 6.3) porosity increases, from the top of the wall to depth, 16% over 1.5 m distance and 32% over a 2 m distance (averaging 16% per

metre). Although porosity is shown to vary it appears that there may be some predictability based on where the relic fresh water lenses may have been if it is to be assumed that the region of highest porosity in figure 6.3 is where formally the top of the fresh water mixing zone may have been found during the time this site was flooded.

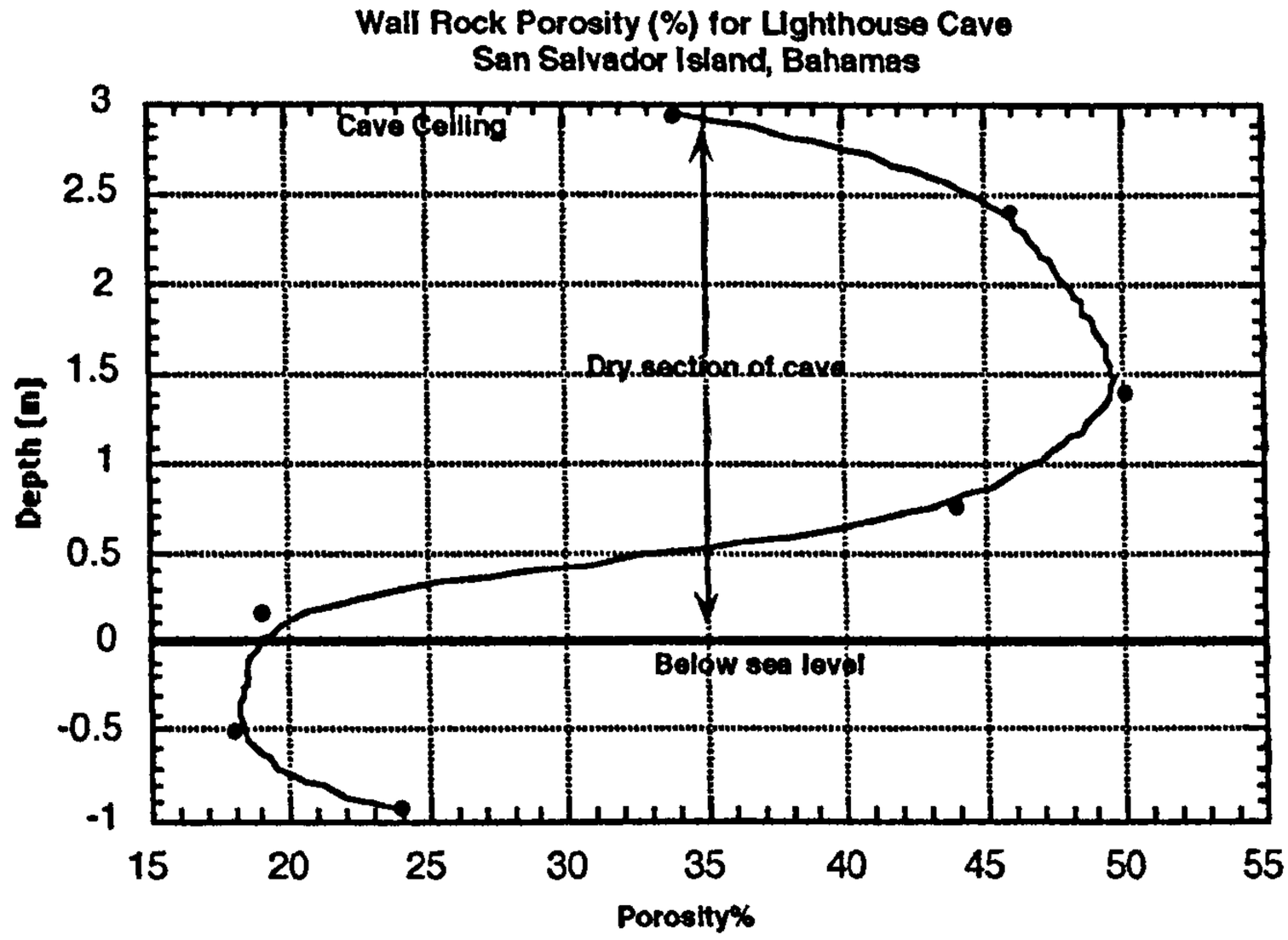


Figure 6.3: Wall rock porosity (%) measured in Lighthouse Cave, San Salvador Island, Bahamas.

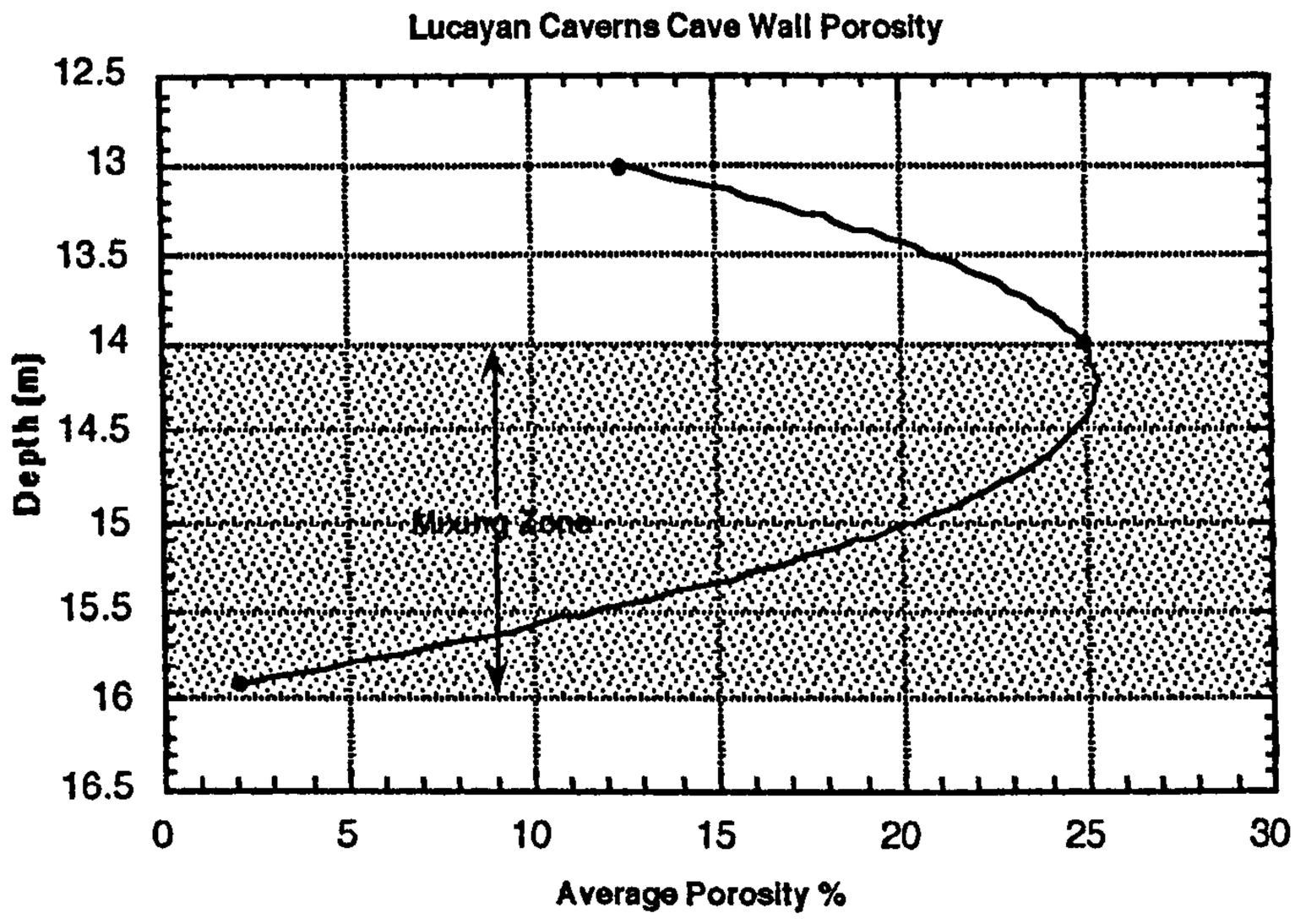


Figure 6.4: Average wall rock porosity from the Lucayan Cavern site from results from figure 5.15.

Based on the fact that dry relic caves harbour a geological history of varying porosity and the current flooded blue holes also harbour the same porosity variations, it may very well be possible that a similar history of porosity could be found at greater depths potentially influencing modern day current flows through the carbonate islands and thereby influencing the configuration of the fresh water lens and associated mixing zone.

6:1:2 Dissolved Oxygen

Dissolved oxygen (DO) concentrations are the highest within the top section of the water column and the lowest recorded DO within the marine section (Figure 4.16 (C), 4.42 (C) and 5.30 and Table 6.1). The range of DO within the blue holes was from (0-6.93) whereas the average for example, for the Salina aquifer was 1-5 mg/l (Shi et al., 1999). Their lower levels of DO may be explained partially as a result of the higher levels of organics found within the aquifer and the probable associated microbial activity one would expect with the available organics. At all three sites in the Bahamas, the DO concentrations within the fresh water lens are already depleted on average by about 33%. However, as seen in the Lucayan multi-oxygen profile (Figure 4.16 (C), DO concentrations increase deeper into the cave away from the entrances. A reason for this may be that further back into the cave passage, organic material which is laterally transported into the cave via tidal flow may not be as readily available for microbial degradation as it is at the entrances of the cave. The amount of organic material being transported in via meteoric transport may not be substantial enough at the sampling time to increase DO consumption. This may however not be the case during the rainy season. Dry season verses wet seasonal studies still need to be done.

Table 6.1: Minimum-maximum ranges of physical, chemical, and biological parameters at three blue hole sites. Concentrations in mg/l, except salinity (g/l), pH (units), temperature (°C), acetate (μM), lens thickness (m), and total bacterial counts (counts/ml), percent dividing cells (per/ml), on and off particles (per/ml).

Parameter	Lucayan Caverns	Owl's Hole	Stargate Blue Hole
Salinity	0.4-35.0	0.4-36.1	5.0-36.5
pH	7.80-8.36	6.92-7.48	7.17-8.70
Temperature	23.6-24.2	23.4-25.9	23.1-27.4
Dissolved Oxygen	0.25-6.17	0.42-6.93	0.0-6.14
Acetate	0.5-34.0	0.0-22.2	0.0-29.0
DOC	0.0-40.0	3.6-41.0	6.0-52.0
POC	0.0-1516		0.006-0.123
Alkalinity	161-236	165-266	146-281
Lens thickness	11.4	15.0	15.5
Total Bacterial population	25118-218776	10232-218776	3890-158489
Percent dividing	<1->15	<4->31	<3-<15

cells			
Bacteria on Particles	0-450	4-87	0-181
Bacteria off Particles	192-368	280-439	185-582

In Owl's Hole, DO does not increase with distance into the cave. It may be possible to explain these results, if DO values are considered a direct result of organic availability in these systems, that the Owl's Hole entrance is itself not in close proximity of a mangrove creek and therefore its' large amounts of available organic material. Mermaid's Lair entrance on the other hand is surrounded by mangroves and although at this time multiple DO profiles are not available for this section of Owl's Hole, it is possible that the available organic material has already been consumed before it reaches the Owl's Hole section of the cave almost 1 km inland; keep in mind that the measured distance on land between the Mermaids and Owl's Hole opening is a great deal shorter than the maze passage distance between Owl's and Mermaid underground. Notable organic input deeper into the cave system is not visually recognizable, i.e., tree limbs, mangrove leaves etc. Extensive grazing by large numbers of macrofauna, i.e., copepods, ciliates, and shrimp, may be responsible for limiting surface source organic input further into the system, and as a result may also reduce the potential influence on the DO supply.

Large amounts of POC found deep within the cave system are mostly autochthonous. Bacteria are responsible for most of the production of the visible POC (Figure 4.28 and 4.29) whereas near or at the entrances of these cave systems a larger portion of the POC arrives via the surface in the form of decomposing leaves and wood. Increase of visible organic material is also noted deep in the interior of the Lucayan Caverns system and most likely a similar explanation for its presence is reasonable.

Stargate blue hole DO profile is not unlike the two other sample site. Like Lucayan Caverns and Owl's Hole, the DO values in Stargate are highest within the upper section of the water column (Figure 5.30), however, unlike Lucayan Caverns and Owl's Hole (Figure 5.5; 4.40), DO values decrease in the south passage, away from the entrance. This is interesting because the passages, north and south, are virtually void of visual organic material except on the floor at 87 m. In Stargate only a light dusting of a gray fine sediment, most likely a mixture of liberated limestone from the surrounding walls and detritus which finds its way into the system via the entrance, can be found on the floor. The water in direct contact with the ceiling is loaded with autochthonous organic material. Whether this amount is significant enough to account for the DO concentrations decreasing was not determined. Most likely, Stargate is a victim of its distance from the immediate input of fresh organic laden marine water. Stargate is a long distance away from either ends of the full length of the fracture

system and by the time the water arrives in Stargates's passages, most of the organics have been consumed and only meteoric input from above provides any bolus input of material.

The Stargate system however, being a fracture system with multiple openings which begin offshore from North Andros and continue to the offshore banks off of South Andros, a distance of nearly 150 miles, stands to be a challenge when addressing some of the potential influencing factors on the water chemistry. To the north and the south, openings are being used by local populations for sewage, water, recreational swimming, and garbage dumps. Although there is water movement along this system the damaging influences human pollutants have on Stargate from other blue holes along the fracture is yet unknown. Future trips to Stargate will entail testing the water for total coliform, fecal coliform. Based on recent outbreaks of gastroenteritis in populations using these watering holes for consumption, human pathogen counts are expected to be high.

In the Stargate hydrolab profile (Figure 5.30), just beneath the LMB from 26 m to about 33 m, is a small zone where DO values increase by almost 1 mg/l vertically, before rapidly decreasing again (Figure 5.30). In the late 80s', the geochemical measurements made by Whitaker (1992) were similar but were thought to be erroneous. However, the jump in DO value is real and presents an interesting question as to what is going on at this particular depth geochemically and biologically. In the entrance within this depth region are wispy clouds of what appears to be bacterial material. When disturbed, these clouds dissipate and usually are re-establish within 24 hours. It has just recently been discovered (Fenchel and Glud, 1998) that the marine sulphide-oxidizing bacteria *Thiovulum majus* has the ability to form characteristic white veils on or above sulphidic sediments. Whether the veils which are seen within the water column may serve a similar function must still be investigated. However, like Stargate, Fenchel also said that he has seen these veils within the water column above sediment (personal communication). Their findings suggest that the white veils serve to increase the flux of oxygen by convective water transport or by swimming to the microbial community of which *T. majus* is part. *T. majus* are among the fastest-swimming bacteria (Fenchel and Glud, 1998) known (150-600μ ms^{-1})[5] The bacteria produce mucous threads to hold them close to the sulphide-containing sediment, with the bacterial cells clustered on the side with the higher oxygen level . The refined architecture of the veils even include holes about a third of a millimetre in radius that allow deoxygenated water to be released. *T. majus* are able to do this by generating convective oxygen transport through the 0.5 mm-thick water layer above the veil at rates that are about 40 times higher than molecular diffusion. Whether the increase in DO is a direct response to chemosensory behavior of bacteria is something which needs to be investigated further. The depth at which this cloud forms is a transition zone

between relatively highly oxygenated water for this site and the boundary of H_2S-containing waters. These veils have been observed (in the context that they have been originally described), on top of very thick layers of purple bacteria found within a cenote, containing highly stratified bodies of water.

What has been observed just recently is that DO levels (6.0 mg/l), within the upper sections of the water column within an open cenotes (297 m in diameter), are about the same as what is found within cave systems with a closed roof (6.14-6.97 mg/l). This was a surprise because it was assumed to a degree that DO level within the fresh water lens was consumed in part during its movement through the ceiling rock. Obviously, this is not exclusively the case. The open cenote (known as the Black Hole) GPS 23° 58" 39' N and 077° 42" 06' W, located 12 nautical miles in from the Deep Creek entrance on South Andros, is a system which is completely isolated from surface connections to any other body of water. At depth however, some exchange of water is possible but minimal based on the highly stratified nature of the water column. The surface is pushed around by winds which are quiet strong generating waves and yet, the DO levels were no higher than 6.0 mg/l. In a cave system such as Lucayan, Owl's Hole or Stargate, this is not possible because it is all sheltered; like an ocean with a lid. Levels are the highest within Owl's Hole (6.97 mg/l) but in Lucayan and Stargate, the levels are almost identical to the Black Hole. Therefore, DO levels within the Black Hole must be in part affected by microbial activity and geochemistry. The salinity of the upper 17 m began at 12 g/l and not 0.5 g/l as seen within Lucayan and Owl's Hole. Based on the higher temperature, higher salinity, calculations show that the water in the Black Hole was only about 15.5% undersaturated.

6:1:3 pH

Between the three sample sites, the pH values ranged from 6.92 to 8.70 (Table 6.1), a broader range than for example the pH values recovered from an aquifer in Salina, Kansas where pH measurements ranged between 7.0-7.5 (Shi, et al., 1999), however, their sampling intervals were every 0.6 m instead of every 0.2 m as it was measured in the Bahamas. It is therefore possible that an sudden changes over very short distances, which have been seen to occur in the blue holes may not have been detected in the Kansas site. In the Bahamas, variations do occur between different sites within the same blue hole cave systems. Whether pH values vary during different seasons or change based on organic influx into the system or other geochemical or biological reasons, is yet unknown.

One feature of particular interest which was noticed while using the hydrolab, and also during manual sampling, was when moving through the major density

interfaces with the hydrolab, pH levels decreased sharply over a few centimetres, (Figure 4.10 and 4.11) for example from 7.18 pH to pH 6.9. In a cenote on South Andros, (The Black Hole), over a 1 m distance the pH went from 8.6 to 6.4, a change of 2.2 pH units. pH drops were also seen when sampling method using the water tubes were used (Figure 4.37, 4.9, 5.24). Here we saw pH values drop, although not as abruptly as with the hydrolab, from pH 7.46 to pH 7.32. Also, the peaks were much more dramatic at the UMB than the LMB. This similar pH change has also been observed in cave systems in the Yucatan (personal comm., Tom Illiffe) where the transition through the UMB had the most dramatic change. The changes that were seen in Mexico were much more dramatic, literally changes of 3 to 4 pH units (alkaline to acidic). Although for those who work only with pore water from sediments, may not find these pH changes significant however, keep in mind that we are recovering this data from a water column which is moving and potentially to some degree mixing. The only explanation for these pH changes, which may apply to this environment, is that the boundaries between the fresh water and saline zone never reaches equilibrium however this does not explain why the low pH values are there to begin with. In the cenote (The Black Hole) pH changes can be explained by a very dens, literally black out layers, of purple bacteria which are found within this 1 m section of water which are most likely producing metabolic acids responsible for the pH changes.

According to Whitaker, extremely high PCO_2 levels exist at these interfaces (Whitaker, 1992) potentially explaining the acidic conditions. This may explain acidic conditins in blue holes like Lucayan where the water column does not have an obvious dense microbial population. The swiss cheese feature of the cave wall (Figure 4.2) in contact with this body of water would support a long term presence of low pH conditions and not a freak event. One thing for sure, this pH change is not exclusive to one cave but seems to be a feature of caves which house bodies of water with varying salinities and have not been found in caves which contain a homogenous water column of like salinity.

A possible explanation for the UMB peak being larger than the lower one may be that the stratified layer at the UMB is more geochemically stable as a result of not being in direct contact with a moving and more dense body of water, i.e., the marine section. As the marine water moves past the LMB, friction is evident as small swirling layers, which can be observed when artificial light is passed through the water layers above and/or below the LMB. This friction, resulting in mixing, serves to prevent the LMB from developing an intense concentrated geochemical gradient. The UMB on the other hand is not directly in contact with a more dense moving body of water, leaving the UMB mostly undisturbed however with still enough water movement not to allow equilibrium. The salinity (Figure 4.16 (A), 4.42 (A), and 5.19) and temperature measurements (Figure 4.8, 4.16 (B), 4.42 (B), 5.2, 5.21, 5,22, 5.23) also correspond

to the fact that the transition through the UMB is sharper than the transition through the LMB.

6:1:4 Temperature

Temperatures at the three sites ranged from 23.1-27.4 °C. The fresh water lens is always 2 to 3 degrees cooler than the underlying marine section (Table 6.1). These numbers will fluctuate by about 1 to 2 degrees from winter to summer. Overall, the temperature is known to stay the same year. This is not the case for all blue holes. Some systems, with their opening unprotected by shade, will reach temperatures of 38 °C to 40 °C (personal observation) during the summer months and than cool by about 10 °C during the winter months. These are generally cave systems which have developed extensive microbial layers that are very dense and generally ink-black or dark brown in colour. It was initially thought that these black to dark brown layers helped to generate higher *in situ* temperatures however, this does not always seem to be the case. It appears that the dense microbial layers which are in many cases responsible for the colour of black holes are hot because of microbial metabolic activity. Continues measurements were taken over several days and a temperature fluctuation of only 5 °C occurred in the top 5 m of the 17 m section above the hot layer. The temperature in the dense microbial layer did not change nor did the cooler layer beneath.

Temperature range is important to bacterial growth because it affects the rate of biochemical processes that are vital to the successful survival of microorganisms within a given environment. The consistent, year-round temperature range within these blue holes would most likely play an important role in the ecology found within these submerged cave environments. So in trying to explain temperature changes within any of these caves systems, it needs to be taken into account whether there is water movement, is there a direct or indirect hydrological connection to a larger body of water which may be feeding the system warm bank waters or warm creek waters, is there obvious microbial activity and most likely other feathers which at this time have not been recognized or addressed.

6:1:5 Organics

According to Whitaker (1992), organically mediated processes appeared to be an important and potentially dominant control on dissolution within the fresh, mixing, and saline zone. The combination of microbial generated CO_2 and H_2S generate dissolutional potential.

Retention of suspended organic matter in the form of POC, is directly proportional to the salinity gradient (Figure 6.5 and 6.6). This was shown to occure without a doubt in the cenote, The Black Hole were measurements went from about 1 mg/l to 17 mg/l at the major densitiy interface. The source of organic carbon, (0 to 1.516 g/l) in blue holes compared to (18 to 53 g kg^{-1}) measured from the Kansas site (Shi et al., 1999), arrives in the caves via the entrances of blue holes, lateral input via tidal marine water and through bacterial activity as already discussed in the DO section (6:1:2). Another source, although understood at this time to be small, is organic material locked within composite grains making up the surrounding rock. Supporting evidence that bacteria are potentially tapping into this organic resource is supported by the high bacterial counts (1,584,893 cells /0.631g/cm^3 rock) for example, found within the first 2 cm of wall rock (Figure 5.15 and 5.16). Carbonate sediments on the floor of the cave, which are dislodged from the surrounding walls, contain some residual carbon (Figure 4.32). Most likely the carbonate sediment from the floor came from the slow dissolution of the ceiling and wall rock and by the time the sediments accumulated on the floor, the available carbon would have been spent by the resident bacterial population found in the wall rock.

Low DOC values measured within Lucayan Cavens (0-40 mg/l) and Owl's Hole, 3.6-41.0 mg/l, (Figure 4.43 and 5.6) may be explained by the small number of restricted entrances to Lucayan Caverns and Owl's Hole. Lucayan Caverns and Owl's Hole concentrations of DOC are considerably lower than those of Stargate, 6-52 mg/l, (Figure 5.31) which in a fracture blue hole system may derive DOC mainly from gross organic input via many cave entrances along it 150 kilometre length. However, according to Whitaker (1992), results showned no significant differences between large cavern openings and small ones, suggesting that organic matter concentrations are not solely dependent upon the presence of cavernous entrances. According to the earlier described DO profiles away from the cavernous entrances, it is felt that entrance numbers and size are important in influencing geochemical perimeters within at least the front of the cave's system.

Evidence of organic mineralization is elevated PCO_2. According to Whitaker (1992) CO_2 concentrations were the highest near the upper section of the MMZ where levels reached 0.98%, more than 30 times higher than atmospheric PCO_2. In the presence of dissolved oxygen decomposition occurs by aerobic oxidation, however, as this process depletes the dissolved oxygen reserves, conditions become anoxic and decomposition proceeds by sulphate reduction.

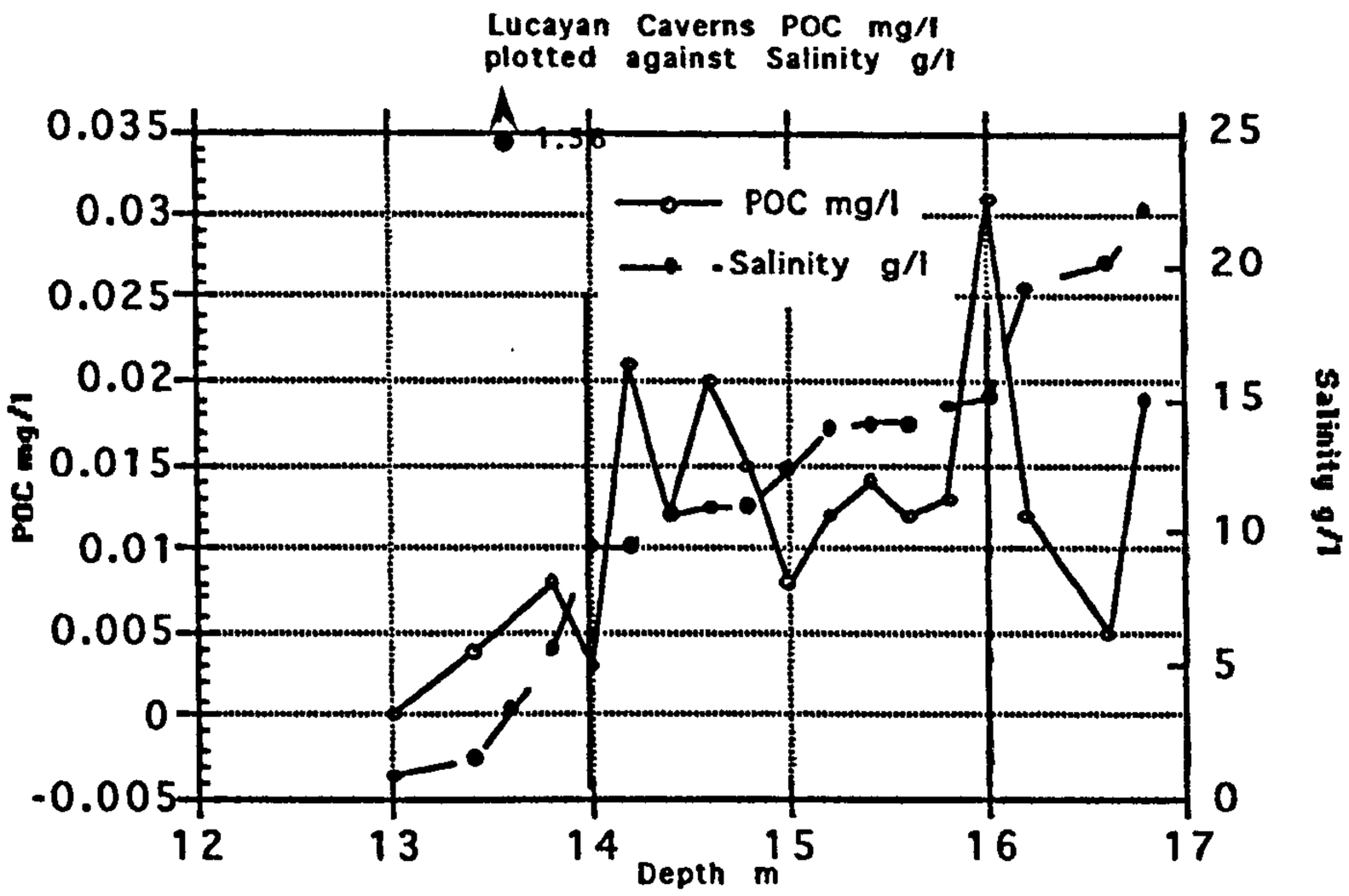


Figure 6.5: Particulate organic carbon (POC) plotted against salinity (Lucayan Caverns)

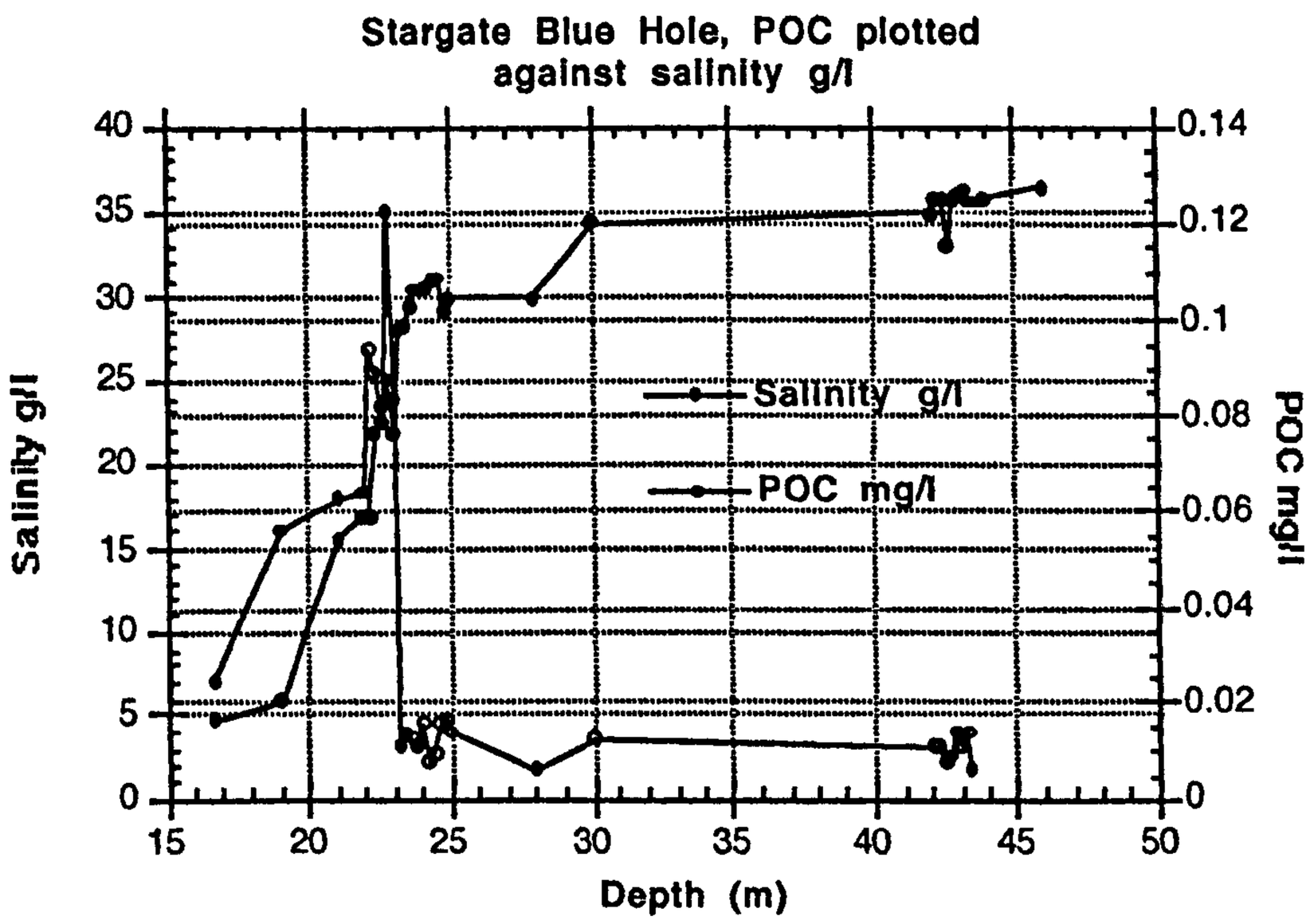


Figure 6.6: Particulate organic carbon plotted against salinity (Stargate Blue Hole)

Processes such as sulphate reduction through the dissociation of H_2S, generate dissolutional potential for the surrounding wall rock. In the presence of oxygen, the mobile agents from mineralization concentrate at the oxic and anoxic or suboxic interfaces within the lens or mixing zone. These water conditions would account for the resulting extreme dissolution seen in the wall rock of the cave where this section of

the wall rock is constantly in contact with this particular section of the water column (Figure 4.2).

With the total acetate levels measured in water samples from all three study sites it could be shown that Lucayan Caverns (Figure 4.18) had the highest concentration of 5 μM. Acetate is highly significant as both a major end-product of fermentation and as substrates for terminal carbon mineralisation, providing a principal substrate for sulphate reduction and methane production in fresh water and anaerobic digesters. The maximum total acetate concentration in sediment from Southdown, Tamar Estuary UK, was about 22 μM (Wellsbury and Parkes, 1995) and are generally less than what is found in the water column within these three study sites. However, sulphate reduction rates are less to non-existent within the water column if compared to Tamar sediments (Parkes et al., 1989). Sulphate reduction rates are low and no evidence of methane production was found in any of the three study sites, possibly explaining why acetate levels are as high as they are within the water column, i.e., the bacteria, which would use the acetate as an energy source, are not there or are inactive. There is possibly another explanation for the presence of high levels of acetate. The ceiling rock where meteoric waters find their way through into the cave house within them an environment not unlike the conditions one would find in sediments or in the water column but on a more condensed scale. The top section of the ceiling rock is the surface on which we stand. This would be relatively well oxygenated. Once beneath the surface, the oxygen supply would decrease along with the availability of gross organic material however shorter chain carbons would be more prevalent although it would be expected that microbs within the ceiling rock would be utlizing this material. This environment would continue to change right up into the capillary zone or the water table. Bacteria within the ceiling rock would continue to mineralize organic material and at some stage, perhaps during a rain storm, this material including bacterial cells could be washed into the fresh water lens and potentially account for higher levels of acetate or other low weight carbon compounds. As we have seen, the water column itself has an environment similar to the ceiling rock, starting off with relatively oxygenated water, higher levels of bacteria and organic material and with depth level drop off till potentially, depending on the location and the nature of the cave system, anoxic conditions are reached. This cycle, on a different scale and under different conditions, would again potentially repeat itself within the sediments found on the cave floors within the cave system.

According to Christensen and Blackburn, (1982), 84% of the acetate in the sediment may not be bioavailable even though total acetate values from Stargate Blue Hole indicate levels of bioavailable acetate up to 29 μM at 22 m (Figure 5.33). This elevated level of acetate correlates with high levels of DOC (Figure 5.31) and high POC (Figure 5.32). Also in Stargate Blue Hole, bacterial populations are not high at the 22

m depth (Figure 5.40). The cell count at that depth was only 25,118 / ml (Figure 5.40) whereas at a slightly deeper depth of 23.8 m the cell count was the highest at this site with 125,892 cells / ml. At 22 m, DO concentration (Figure 5.30) is still at 3 mg/l (62% undersaturated by DO). It is possible that there is not enough DO to support the heterotrophic bacterial population at this depth and the water is still too oxygenated to allow sulphate reduction to occur. However at 22 m ^{35}S reduction was almost nill at 0.02 nmol/ml/day for the non-filtered sample (Figure 5.36) and 0.3 nmol/ml/day for the filtered samples (Figure 5.37).

6.2 Radiolabeled Compounds

6:2:1 Rates of Acetate Turnover and Sulphate Reduction

Acetate turnover rates in the Lucayan Caverns (0 to 4.8 μM) was overall much higher than either Owl's Hole (0 to 0.76 μM) or the Stargate site (0 to 0.31 μM) (Table 6.2 and Figure 6.7). For comparison acetate turnover in the mudflats of the Tamar Estuary ranged from 0.39 to 0.35 μM (Wellsbury and Parkes, 1995). Maximum turnover rate within Lucayan Caverns occurred below the LMB at 16.8 m (Figure 4.18) with a value of 34 μM. Another peak of 15.5 μM was found at the LMB which was slightly higher than one found in the fresh water lens of 12 μM at 13 m. At the LMB and the UMB, PCO_2 levels range from 0.4% to 0.45% (Whitaker, 1992). This corresponds with zones of high DOC levels (Figure 5.31). The high levels of PCO_2 within the middle to upper part of the mixing zone can be explained by mineralization of organic carbon by heterotrophic bacteria.

Table 6.2: Minimum-maximum concentrations of bacterial activity. Rates in (μM/hr) for acetate, (μM/day) for bicarbonate, (nmol/ml/day) for sulphate, and (cells/ml/day) for thymidine.

Radiotracer	Lucayan Caverns	Owl's Hole	Stargate Blue Hole
^{14}C Acetate (2hr)	0.0-4.1	0.0-0.4	0.0-0.31
^{14}C Acetate (6hr)	0.0-4.8	0.0-0.76	0.0-0.09
^{14}C Bicarbonate (2 day)	23.0-79.0	0.5-28.0	0.0-6.8
^{14}C Bicarbonate (5 day)	5.0-33.0	0.0-4.9	0.0-1.5
^{35}S Sulphate (2day)	0.0-3.5	0.0-3.4	0.0-0.04
^{35}S Sulphate (5day)	0.0-1.8	0.0-0.5	0.0-0.0
^{35}S Sulphate (2day)			0.0-.365

Filtered		
^{35}S Sulphate (5day)		0.0-0.14
Filtered		
[^{3}H] Thymidine (15 min.)	0.5-3.8x10^{5}	0.0-8.1x10^{5}

Although elevated PCO_2 levels can be found in the MMZ at all three sites, maximum acetate consumption occurs in different sections of the water column. In Owl's Hole maximum activity occurred above the UMB for both the 2 and 6 hour incubation times, and in Stargate just above the LMB, the largest consumption rate was around 42 m. It may very well be possible that CO_2, which is generated below either of the major density layers, becomes trapped and that the high values do not necessarily reflect bacterial activity.

Acetate is the dominant substrate for sulfate reduction in marine sediments (Parkes et al., 1989; Sørensen et al.,1981), and should reflect sulfate reduction rates (i.e., 1:1 relationship). In Lucayan Caverns this correlation is not so clearly seen as in Owl's Hole where peak acetate turnover rates within the water column associate with sulphate reduction rates. The same is seen within the Stargate system although of the three sites, Stargate had the lowest

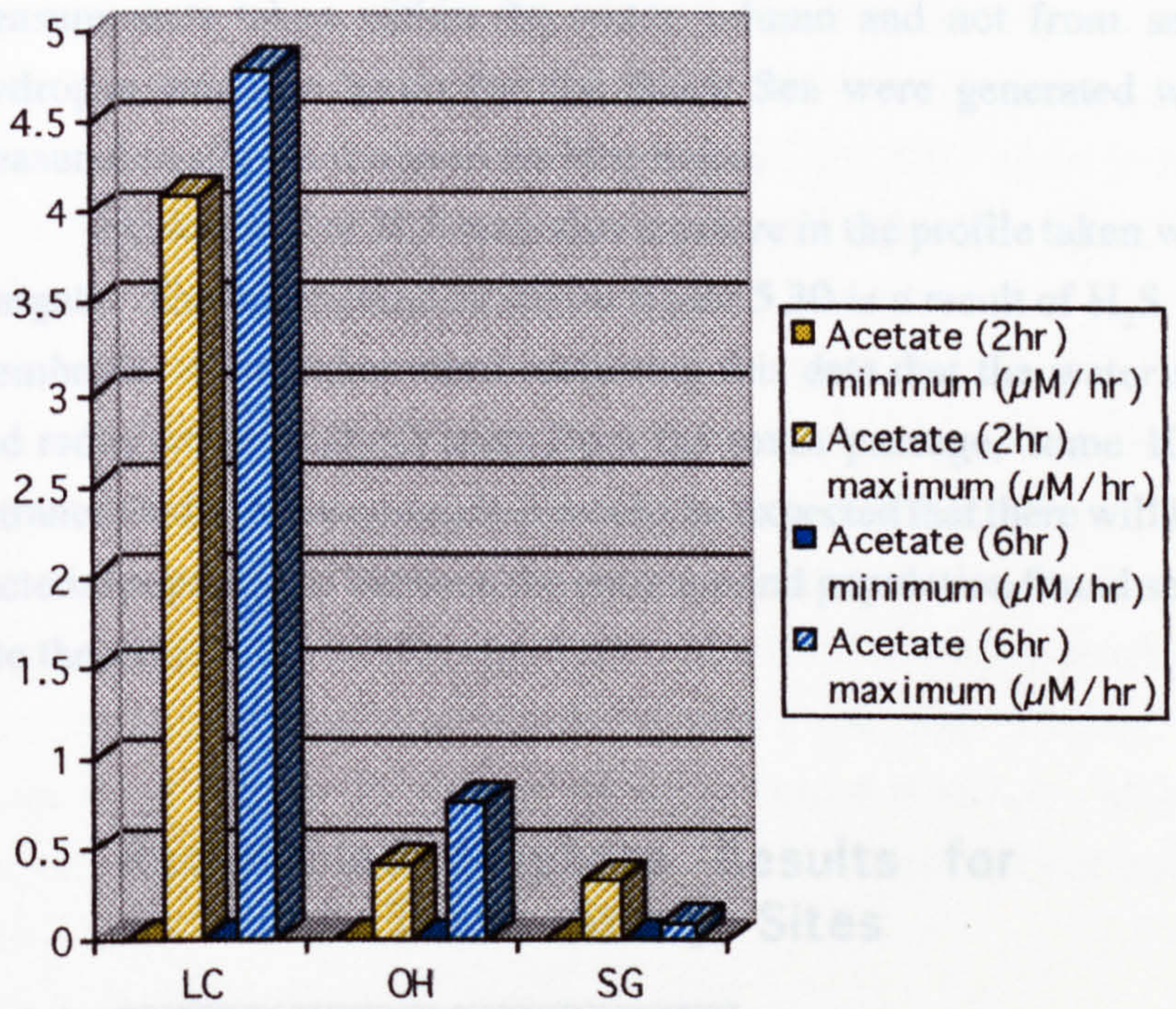


Figure 6.7: Bar graph showing minimum and maximum activity for ^{14}C acetate consumption in Lucayan Caverns (LC), Owl's Hole (OH) and Stargate Blue Hole (SG).

sulphate reduction rates (Figure 6.8) for nonfiltered samples, however, the filtered sample results (Figure 6.9) for sulphate reduction prove that Stargate has the highest turnover rate. This is not surprising because of the presence of an extensive anoxic region within the lower section of the water column. A concentration of H_2S, > 0.25 mg/l was measured from the anoxic section of the water column (one measurement taken using Hach chemicals just to see if H_2S could be detected. Hydrogen sulphide levels do not appear high in the blue hole samples if compared to sediment and or water levels produced in the lab for example from Sorokin's work in the Black Sea were levels were produced on a daily based of 2 mg /l from sediments and within the water column, 7.1 mg /l from samples collected from a 260 m depth. Levels higher than 2.1 mg/l have been measured in cenotes *in situ* on South Andros within the water column (personal observation). Artificial wells dug in the bottom of the Obanul de la Movial sinkhole in Romania (Serban and Popa,1992) revealed H_2S levels within the water as high as 41.2 mg/l. However the source of H_2S is not bacterially mediated. Based on $\delta^{34}S$ values, the H_2S was coming from a magmatic source. This cave system is known

to be positioned, based on geomagnetic anomaly recordings, ontop of hot magmatic rocks located at the base of the Dobrogean limestone strata (Sarbu and Popa, 1992). What needs to remembered here is that H_2S result from the blue hole sites come from measurements taken within the water column and not from sediment pore water. Hydrogen sulphide levels for the Black Sea were generated within a lab and not measured *in situ.* as it was in the blue holes.

Evidence of of H_2S was also measure in the profile taken within the entrance of Stargate. The line marked as (B) on figure 5.30 is a result of H_2S poisoning of the DO membrane. Remember when reviewing this data that the water samples for bacterial and radioisotope analysis came from the south passage, some 100 m away from the entrance shaft. Most certainly it would be expected that there will be some difference in bacterial populations between the entrance and population found several hundred metres into the cave.

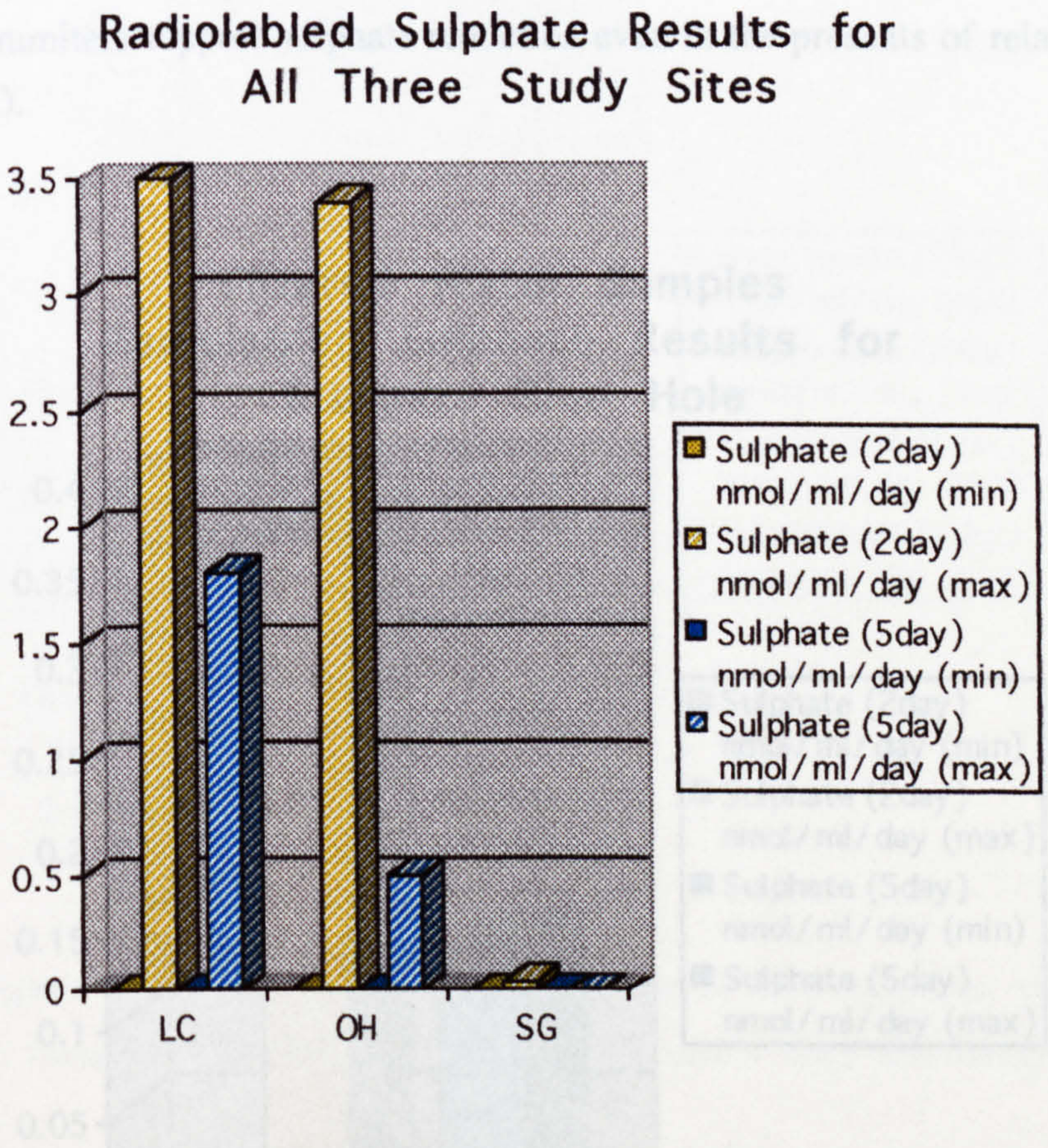


Figure 6.8: Bar graph showing minimum and maximum sulphate reduction rates for Lucayan Caverns, Owl's Hole and Stargate Blue Hole. These are non-filtered results.

Sulphate reduction rates were slightly higher (0-3.5 nmol/ml/day) in the non filtered samples (Figure 6.8) than the filtered samples (0-0.14 nmol/ml/day) suggesting

that sulphate reduction may also be occuring within POM within water samples but is certainly not occurring exclusively within POM as figure 5.37 illustrate. The reason for filtering the sample was to try to answer the question as to why sulphate reduction was occurring in samples collected from 15.5 m to 26 m that contained DO values in excess under conditions where sulphate reduction are known to take place. Speculation was that sulphate reduction was occurring in POM and filtering the samples would eliminate these higher values. Results (Figure 5.37 and 5.36) do illustrate that some sulphate reduction is most likely occurring on POM.

There is however the situation where sulphate reduction appears to be occurring in the section of the water body where microbial populations are at it highest. In the Black Hole cenote sulphate levels dropped from 2900 mg/l to 2700 mg/l. The drop in 200 mg/l occurred at the maximum peak of sulphide measurements of 2.2 mg/l before returning to almost zero levels. This is also the exact point at which temperatures rises from 29.5 °C to almost 36 °C. DO levels crash from 5.8 mg/l to 1.2 mg/l, salinity jumps from 12 g/l to 29 g/l, and pH drops from 8.6 to 6.4. Maybe these geochemical peramiters support sulphate reduction even in the presents of relatively high levels of DO.

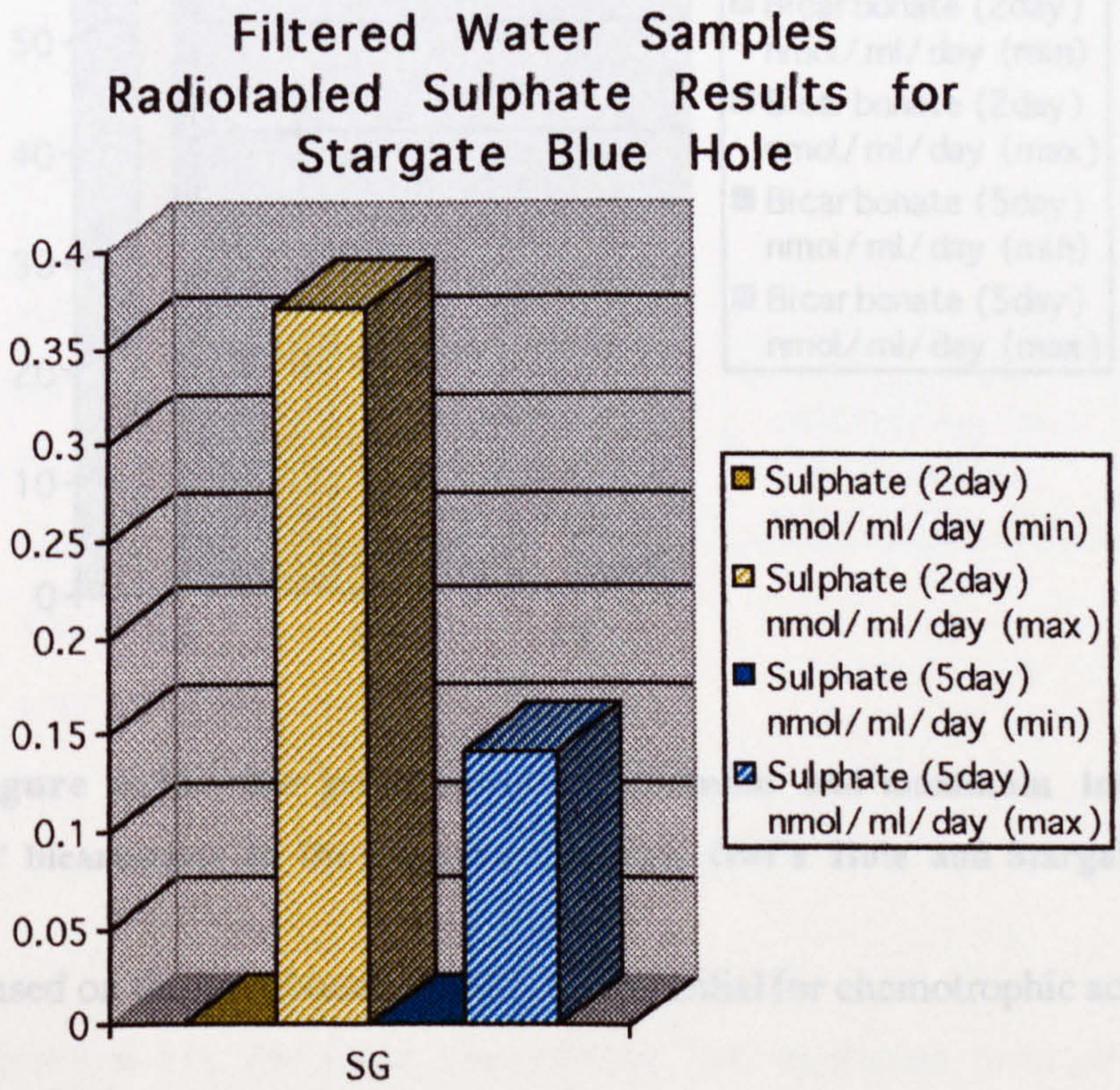


Figure 6.9: Sulphate reduction rates for samples which were filtered prior to inoculation.

6:2:2 ^{14}C Bicarbonate

Positive results for chemotrophic activity have been obtained at all three sites (Figure 6.10 and Table 6.2). The 2-day incubation rates for Lucayan Caverns are twice as high as the other two sites (Figure 4.20) and the 5-day rates are approximately 4 times higher than the Owl's Hole values (Figure 4.46) and approximately 14 times higher than Stargate values (Figure 5.35). In Lucayan Caverns, peak activity appears to occur within close proximity of the UMB and the LMB. In Owl's Hole, there was only one major area of peak activity and that occurred above the UMB. Stargate, on the other hand, had also peak activity as seen in Lucayan Caverns near the LMB and another in the same vicinity where maximum sulphate reduction was occurring.

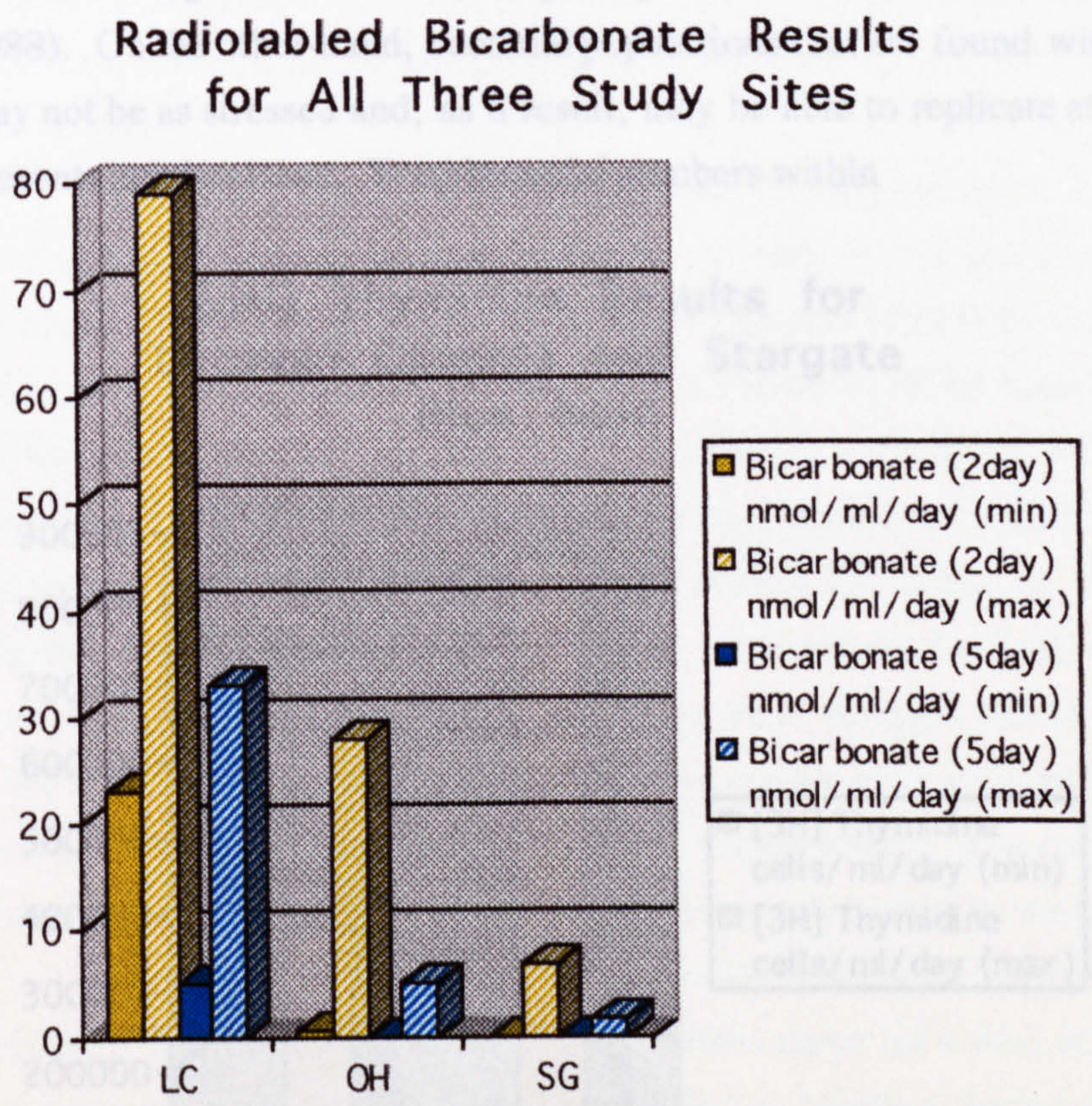


Figure 6.10: Bar graph showing minimum and maximum incorporation rates of ^{14}C bicarbonate in the Lucayan Caverns, Owl's Hole and Stargate Blue Hole.

Based on the bicarbonate results, the potential for chemotrophic activity exsists.

6:2:3 [^{3}H] Methyl Thymidine

Thymidine results (Figure 6.11) indicate that the bacteria within Stargate are active metabolically and are synthesizing DNA. Tritiated thymidine is taken up only by dividing bacteria from natural environments (Fuhrman and Azam, 1982; Mården et al., 1988). Thymidine results demonstrate that bacteria within Stargate are metabolically more active than in Lucayan Caverns. In Lucayan Caverns one observes signs of bacterial metabolic activty but based on the relatively high turnover rates for ^{14}C acetate, ^{14}C bicarbonate, and sulphate, it appears that some bacteria may be experiencing nutrient limitation, high turnover rates and even the early phases of starvation. It is known, however, that bacteria in an early phase of starvation may still synthesize DNA, although at a low rate and perhaps as the result of DNA repair (Mården et al., 1988). On the other-hand, bacterial populations that are found within the Stargate site may not be as stressed and, as a result, may be able to replicate at a higher rate. This presents another issue. Total bacterial numbers within

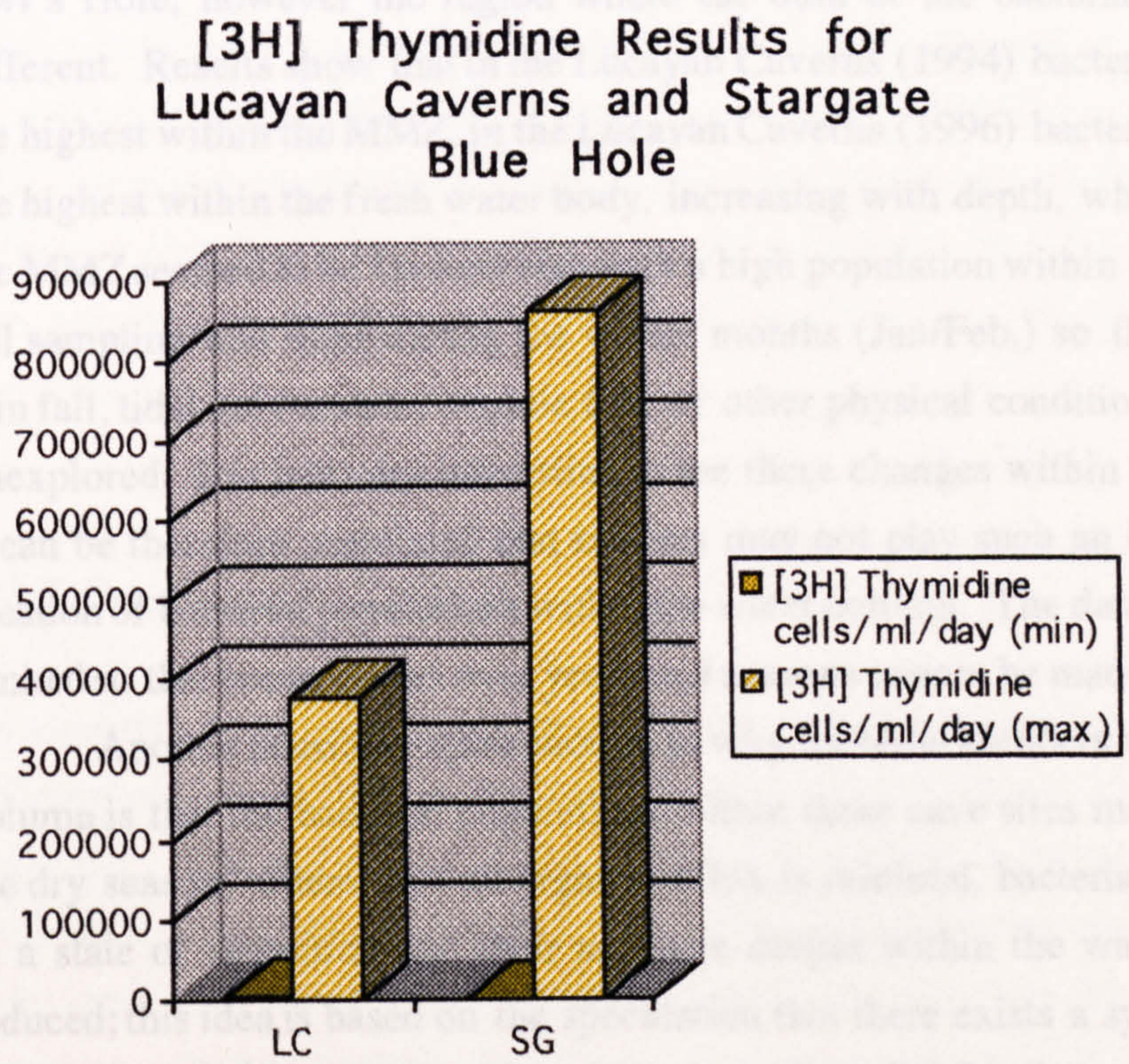


Figure 6.11: Bar graph representing [^{3}H] thymidine incorporation rates for both the Lucayan Caverns and Owl's Hole sample site.

Stargate were lower than at any of the two other sites which suggests active grazing by zooplankton and other ciliates.

6:3 Bacteria

6:3:1 Total Bacterial Counts and Dividing Cells

Lucayan Caverns and Owl's Hole have very similar bacterial counts (Table 6.1) within the water column, 10^5 to 10^6 /ml, but Owl's Hole has the highest number of dividing cells (4-31 cells/ml). In the open ocean, marine bacteria number around 10^7 to 10^9 /ml (Moriarty and Bell, 1993). Results from Stargate demonstrate the sample site to have approximately 6.0 x 10^4 bacterial cells, which is surprising. Surprising because based on the massiveness of this site, the extent of this system, and the potential for a great deal more influx of organic material, a higher bacterial population was expected. The total bacterial profile however, may have been very different if the water samples had been examined from the entrance shaft at Stargate as wall as Lucayan Caverns and Owl's Hole.

The bacterial counts from Stargate may be very similar to Lucayan Caverns and Owl's Hole, however the region where the bulk of the bacteria is found is slightly different. Results show that in the Lucayan Caverns (1994) bacterial populations were the highest within the MMZ, in the Lucayan Caverns (1996) bacterial populations were the highest within the fresh water body, increasing with depth, whereas in Owl's Hole the MMZ seemed to be favored also with a high population within the fresh water lens. All sampling was done during the winter months (Jan/Feb.) so that variations due to rain fall, tidal movements, organic flux or other physical conditions have not yet been unexplored. It is just very interesting to see these changes within the same season and it can be therefore postulated that seasons may not play such an important role in the location of bacterial populations within the water column. The data base for Stargate is limited so that comparisons over years and seasons cannot be made at this time.

Another possible explanation as to why bacterial numbers vary within the water column is that the bacterial populations within these cave sites may be cyclic. During the dry season, where vertical organic influx is minimal, bacterial populations may be in a state of starvation and their numbers deeper within the water column could be reduced; this idea is based on the speculation that there exists a syntrophic relationship between groups of bacteria within these sites. Once POC is introduced into the system via meteoric water, bacterial populations within the fresh water explode. Mineralization of the POC to smaller or other forms of carbon now become available for sequential populations deeper within the water column. At this time the bacterial numbers can expand at depth till the available energy is consumed and the cycle begins again like a parabolic wave. To test this hypothesis the water column would have to be sampled

during or near the end of a dry season and sampled again within 24 hours after a substantial rain fall.

The large numbers of bacteria found within the water column are not an indication of a necessarily healthy population. Bacteria which may be in a state of starvation will respond by rapid division and the formation of small cells (Morita, 1992). Therefore, numbers of bacteria may increase during starvation and, conversely, they may decrease when food is plentiful (Moriarty and Bell, 1993) because of grazing by zooplankton and other troglodytic animals which are known to be quite numerous within these blue holes.

So it is possible that the large numbers seen within Lucayan Caverns are not well-fed but rather responding to oligotrophic conditions, whereas in Stargate the population is smaller as a result of significant amounts of grazing. The thymidine results indicate that bacterial populations are actively dividing and that bacteria are not necessarily in a state of starvation. In Lucayan Caverns, this does not appear to be the case. Thymidine results are not indicating a rapidly dividing population, however bacterial numbers are higher. There is a potential microbial food web within the blue holes that keeps bacterial numbers down. Copepods, according to Bell et al. (1993), do not graze bacteria directly, however, according to the findings by Fosshagen and Illiffe (personal communication) troglodytic copepod species are found within the water column at sites where bacterial numbers are the highest. Based on the extremely small size and soft appendages, bacteria are most likely the only food source for this particular species.

6:3:2 Bacteria-On-Particle

By almost three orders of magnitude Lucayan Caverns has more bacteria-on-particle (0-450 per/ml) counts than either Owl's Hole (4-87 per/ml) or Stargate (0-181 per/ml) Table 6.1. The abundance of attached bacteria appears to be related to the amount of particulate matter (refer to Figure 5.43). Bacteria which were attached to particles tend to be larger, but according to Kirchman (1993), do not grow faster than free bacteria, however, attached bacteria do take up organic compounds at faster rates than free bacteria (Simon, 1985; Kirchman, 1993). According to Paerl (1978) autoradiographic studies have shown that a large fraction of the organic carbon taken up by attached bacteria appears in the surrounding mucilage which is interesting for the reason that all the bacteria which were counted as on-particle were attached to a proteinaceous material (refer to section 4:4:1). Attached bacteria have a role in rapidly solubilizing particulate organic matter which can be utilized by the free-living bacteria and may be in part play a role in the high acetate levels measured within the water column.

6:4 Sediments

6:4:1 Mud

The mud deposits found on the floor within Lucayan Caverns and Owl's Hole, and which was identified during this project to be lipidocrosite (Fe_2O_3), originated from atmospheric dust blown up into the stratosphere during dust storms in North Africa (Glaccum and Prospero, 1980; Eaton and Boardman, 1985; and Mann, 1986). Speculation as to how substantial amounts of this material got well into these cave systems is open to debate. Between depositional sequences in the Bahamas some of this same, however highly weathered version of lipidocrocite, can be found as a part of the hard caliche layers, giving it its distinct orange colour. These thin-layer deposits are important stratigraphic tools, as they indicate periods of subaerial exposure and , as such, are helpful in unraveling the depositional stratigraphy of carbonate deposits throughout the Bahamas (Carew and Mylroie, 1985).

Visual examination of these mud deposits *in situ*, and the knowledge that this material is found in excess of 1500 to 2000 m into the cave system, well away from the entrance, suggests that the most likely mode of transport into the cave is water. Further evidence to support this observation are the mud cracks. Based on geological sequences the material must be in place first before the act of drying out is possible. However, one could argue that the material could have been blown into the cave and that large amounts of water could have percolated through the ceiling rock, followed by another drying-out sequence, causing the appearance of mud cracks, but a close examination of the banding within the mud deposits indicate a very gentle depositional history. Drips from the ceiling would have left pock marks and disturbed the fine banding.

The banding found within the mud deposits may be a direct result of bacterial oxidation/reduction activity. The mud deposits are found in nearly anoxic water, and their microscopic examination revealed that bacterial numbers were extremely low (≤1 cell/ml). A review of the literature indicates that numerous microorganisms can reduce ferric iron during the metabolism of organic material (Ehrlich, 1981; Blakemore 1982; Nealson 1983). The rate and extent of ferric iron reduction depend on the forms of ferric iron available (Lovley, 1987). Most of the ferric iron in sediments is resistant to microbial reduction.

Ferric iron-reducing organisms can exclude sulfate reduction and methane production from the zone of ferric iron reduction in sediments by out-competing-sulphate-reducing and methanogenic food chains for organic matter when ferric iron is available as amorphous ferric oxyhydroxide (Lovley, 1987), however, lepidocrocite is

dimorphic and how this changes the ability of ferric iron-reducing organisms' ability to work with this substrate is yet unknown. Currently deposits are being examined for paleomagnatic data which may help to determine the age of the deposits and the caves.

6:4:2 "Mung"

The extensive amount of "mung" sediment found in Lucayan Caverns, Owl's Hole and to a lesser degree, Stargate blue hole, has been identified as a proteinaceous substance based on amino acid analysis following hydrolysis of the mung samples. This material thus far has only been found in inland caves, not marine caves. It is known that proteinaceous material can only be produced by life forms and the only one known to exist within the blue holes of the Bahamas capable of producing such material are bacteria. Also from the results acquired from the media plate experiments (refer to section 4:3:1), it is known that the bacteria which grew on these plates produced copious amounts of slime within very short periods of time.

Of great interest is the discovery that in caves explored in the South American jungle, Yucatan, Romania, Turkey, Hawaii and Bimini, mung material has not been identified (Tom. Iliffe, personal communication). My experience is similar. Cave diving in Australia, Florida and the UK, mung was never observed. Naturally the big question is why. Of all the cave diving sites mentioned, Bimini, Hawaii, the Yucatan, Australia and South America, several common denominators concerning cave environments can be identified; warm temperatures, fresh water lens floating on top of sea water, bacteria in the water column, and except for South America, the Yucatan, Florida, and Hawaii, all have none or very little top soil.

Two land features which are prevalent in the Bahamas and not at any of the other mentioned sites are mangroves, except for Florida, and Bahamian pines. All islands in the Bahamas have mangrove swamps along the coast. All the islands from the middle of the Bahamas, i.e., from Nassau north, have Bahamian pines. Mangrove swamps are of interest to the mung story because they contain enormous amounts of organic material and it is very possible that organic material from the swamp is washed into the caves on a daily bases and is utilized by heterotrophic bacterial communities living within the caves. When examining the Stargate site on south Andros, Stargate is the only cave of the three study sites which is not directly connected to a mangrove swamp. Stargate blue hole is approximately 350 m away from the coast while both Lucayan Caverns are fed hydrologically by a mangrove creek and Owl's Hole has mangrove swamp areas to the south. The Mermaid Lair entrance to the south of Owl's Hole entrance (Appendix 1) is surrounded by mangroves and entering the Owl's Hole cave system via Mermaid's Lair, mung material is again found in large amounts,

however in Owl's Hole, which is approximately 1 mile inland to the north from Mermaid's Lair, the amount of mung is already noticeably less.

Another environmental feature which has been seen associated with inland caves in the Bahamas is tannin-laden waters which are bodies of water either directly in contact or in close proximitly with the cave waters. Lucayan Caverns and Mermaids's Lair have tannin water in contact with the cave water in contrast to Stargate and Owl's Hole where tannin waters have been seen only in cave waters after storm events which produced torrential rain fall.

Unfortunately, at this time the question why the mung material is in Bahamian caves only cannot be answered with any certainty. Some of the more interesting questions concerning this "mung" is why bacteria are producing it, why is there so much of this material; and is it because there is a high production rate or a long residence time? What is known is that small troglodytic crustaceans, known as Thermosbanaceans, use mung as a source of food. Some of this mung material has been found in their gut cavity (personal observation) and most likely further examination of other cave ciliates will reveal further dependence on the mung material as a food source. However, one thing for certain; this mung material plays a major part in making blue hole diving in the Bahamas a very dangerous activity.

6:5 Geology

6:5:1 Rock Cores

Thin-section analysis of the rock cores recovered from the wall rock within Lucayan Caverns and hand samples from Owl's Hole and Stargate indicate that at the sites where samples were collected, the cave walls were composed of bioclastic deposits. The Stargate system is the only site which had a mixture of bioclasts and ooids. This is not unusual in that Andros has some of the largest ooid shoals in the Bahamas. Overall, the predominant grains were bioclastic which supports the theory (Schwabe, 1993) that caves appear to form preferentially in bioclastic deposits. Bioclastic deposits are generally not as well cemented as oolitic deposits and some other inorganic carbonated deposits. Bioclastic deposits have generally a higher percentage of aragonite which is a metastable mineral compared to low magnesium calcite and will dissolve away more rapidly. Another factor which also accelerates dissolution of bioclastic deposits is the higher surface to air ratio because of the extensive ornamentation of the composite grains.

Another interesting find concerning the rock cores was the high numbers of bacterial cells (510,505-1,321,295 cells/gm/cm^3) within them (Figure 5.15 and 5.16). This was a major discovery and an important one when looking for supporting evidence for the hypothesis that bacteria are involved in cave development. Bacterial cell numbers where the highest within the first 4 cm section of all three cores and at 6 cm into the wall, bacterial cell counts were dropping below water column values except in the most shallow sample (13 m). Following the 6 cm section, the rest of the cores had less than the water column at those particular depths. Of particular interest is the finding that the highest bacterial numbers were found from the cores from the UMB with a count of (1,321,295 cell in the first 2 cm directly in contact with the water column). This section of rock had also the highest porosity and therefore the least weight (20-30% porosity). This is the section of the wall which looks like Swiss cheese (Figure 4.2). An argument could be presented that because of the extreme dissolution occurring at this depth, the surface to water ration is higher and naturally with more surface area, more bacteria will utilized the available space. However one chooses to look at this, and I guess it is a bit like the chicken and the egg story, the water chemistry within the mixing zone of the water column is being altered by bacterial presence and maybe irregardless of whether the bacteria are free floating within the MZ or are attached to the associated wall rock, either way the limestone is being dissolved by bacterial involvement. Future studies will involved rock core section where bacterial digestion rates, independent of water column chemistry will be determined.

Therefore, as a result of this study it can be concluded that:

1) all three blue hole systems are physically and geochemically stratified with steep major gradients both at the UMB and LMB,
2) bacterial populations were present in all three sytems (3,890 to 218,776 cells/ml),
3) bacterial populations also varied considerably in the different water masses and at the interfaces described in the vertical water column,
4) bacterial metabolic activity was demonstrated by measuring acetate turnover, sulphate reduction, and autotrophic carbon fixation,
5) based on thymidine incorporation results, bacteria were dividing,
6) rock cores taken above the UMB, at the UMB and within the MMZ, showed bacterial presence up to 8 cm into the wall.

Questions have been posed and answers have been suggested about bacterial involvement in cave development. There are large populations of viable bacteria which are capable of generating CO_2 and H_2S, according to Whitaker's results and are responsible for the high PCO_2 levels found within the MMZ. Suspended organic material is directly proportional to the salinity gradient which influences the location of

bacterial populations within the vertical water column, which in turn effects the water chemistry. Bacteria can be found in significant numbers within the wall rock, releasing CO_2 and H_2S and thereby dissolving substrate, generate large pores which eventually, together with structural failure, produce large cave passages. We also know now that blue hole cave systems are very diverse, no two sites are the same. Examination of certain sites within a system does not reveal what could be found on other locations of the caves and do not predict that over horizontal and vertical distances the cave environment and ecology change. This is extremely important to understand when trying to produce models for diagenesis or geochemical modeling. We do not understand enough about the underground environment to even begin to pigeon-hole conditions for cave developement. The carbonate environment in which these blue holes take shape are formed in virtually 99.9% pure carbonate and still, within these environments, the potential for variations are as numerous as for a land mass with many different lithologies. The quest of trying to understand how, what, and why within the blue holes environment has only just begun.

References

AGASSIZ, A., (1984) A reconnaissance of the Bahamas and of the elevated reefs of Cuba: *Musum of Comparitive Zoology Bulletin* **26**: 1-203

AMY, P. S., HALDEMAN, D. L., RINGELBER, D., HALL, D. H. AND RUSSELL, C., (1992) Comparison of identification systems for classification of bacteria isolated from water and endolithic habitats within the deep subsurface: *Applied and Environmental Microbiology* **58**: 3367-3373

ANDERSEN, K. W., (1990) Bats of San Salvador Island, Occational Paper, No. 1., Bahamian Field Station, San Salvador, Bahamas.

ANDERSON, R. T., CHAPELLE, F. H. AND LOVELY, D. R., (1998) Evidence against hydrogen-based microbial ecosystems in basalt aquifers: *Science* **281:** 976-977.

ANTON, J., I., MESEGUER, AND RODRIGUES-VALERA, F., (1988) Production of an extracellular polysaccharide by Haloferax mediterranei: *Applied Environmental. Microbiology* **54**: 2381-2386

ATKINSON, M., SMITH S. V. AND STROUP, E. D., (1981) Circulation of Enewetak Atoll lagoon: *Limnology and Oceanography*, **26:** 1074-1083

ATLAS, R. M., (1981) Microbial degradation of petroleum hydrocarbons: An environmental perspective: *Microbiological Reviews* **45**: 180-209

AUSTIN, J. A. AND SCHLAGER, W., (1986) Ocean Drilling Program Leg 101 Explores the Bahamas *in* Curran H. A., ed., 3rd Symposium on the Geology of the Bahamas: CCFL Bahamian Field Station 1-33

AYERS, J. F. AND VACHER, H. L., (1986) Hydrogeology of an atoll Island: A conceptual model from detailed study of a Micronesian example: *Ground Water*, **24:** 185-198

BACK, W., HANSHAW, B. B., HERMAN, J. S AND VAN DRIEL, J. N., (1986) Differential dissolution of a Pleistocene reef in the groundwater mixing zone of Coastal Yucatan, Mexico: *Geology*, **14:** 137-140

BACK, W., HANSHAW, B. B., PYLE, T. E., PLUMMER, L. N AND WEIDE, A. E., (1979) Geochemical significance of groundwater discharge and carbonate solution to the formation of Caleta Xel Ha, Quinta Roo, Mexico*: Water Resources Research*, **15:** 1521-1535

BALKWILL, D. L., (1989) Numbers, diversity, and morphological characteristics of aerobic, chemoheterotrophic bacteria in deep subsurface sediments from a site in South Carolina: *Geomicrobiology Journal*, **7:** 33-52

BALKWILL, D. L. AND GHIORSE, W. C., (1985) Characterization of subsurface bacteria associated with two shallow aquifers in Oklahoma: *Applied and Environmental Microbiology*, **50:** 580-588

BALKWILL, D. L., BOONE,D.R., COLWELL, F.S., GRIFFIN, T., KIEFT, T.L., LEHMAN, R.M.,McKINLEY, J. P., NIERZWICKI-BAUER, S.,ONSTOTT, T. C., TSENG, H.Y., PHELPS, T. J., RINGELBERG, D., TUSSELL, B., STEVENS, T.O.,WHITE, D. C. AND WOBBLER, F. J., (1994) D.O.E. seeks origin of deep subsurface bacteria: *EOS* **75:** 395-396.

BARNA, D. K., BHAGAT, S. D., PILLAI, K. R., SINGH, H. D., BARNAH, J. N. AND LYERGAR, M. S., (1970) Comparative utilization of paraffins by a Trichosporen species: *Applied Microbiology* **20:** 657-661

BARNES R. S. K. AND HUGHES, R. N., (1993) An Introduction to Marine Ecology, Blackwell Scientific Pub., 351

BEAUCHAMP, E. G., TREVORS, J. T., AND PAUL, P.W., (1989) Carbon sources for bacterial denitrification: *Advances in Soil Science* **10:** 113-142

BENJAMIN, G. J., (1970) Diving into the blue holes of the Bahamas: *National Geographic Magazine* **138:** 347-363

BERN, L., (1985) Autoradiographic studies on *methyl*-[3H] thymidine incorporation in a cyanobacterium (*Microcystis wesenbergii*)-bacterium association and in selected algae and bacteria: *Applied and Environmental Microbiology* **49:** 233-235

BERNER, R.A., (1968) Calcium carbonate concretions formed by the decomposition of organic matter: *Science* **159:**195-197

BJORNSTAD, B.N., LONG, P. E., AND LORENZ, J.C., (1996) Fractures adjacent to a Miocene volcanic neck, Cerro Negro, Cebolleta Land Grant, New Mexico: *GSA Abstract with Program.*, **28:** 49-50

BJORNSTAD, B. N., GULLETT, C. D. AND LONG, P.E. (1997), Geohydrologic and Geochemical Characterization in Penny, S. Amy and Dana L. Haldeman eds., The Microbiology of the Terrestrial Deep Subsurface CRC Lewis Publishers, NY.

BLAKEMORE, R. P., (1982) Magnetotactic bacteria: *Annual Review of Microbiology* **36**: 217-238

BLOCHL, E., RACHEL, R., BURGGRAF, S., HAFENBRADL, D., JANNASCH, H. W. AND STETTER, K. O., (1997) Pyrolobus fumarii, gen. and sp. Nov., represent a novel group of archaea extending the upper temperature limit for life to 113°C: *Extremophiles* **1** (1) 14-21.

BOSS, S. K., (1991) Bahamian "Whitings" as turbulent-flow phenomena in Bain, R. J., (ed.) Proceedings of the fifth sysmposium on the Geology of the Bahamas, Bahamian Field Station San Salvador, Bahamas.

BOTTRELL, S. H., CAREW, J. L. AND MYLROIE, J. E., (1993) Inorganic and bacteriogenic origins for sulfate crusts in flank margin caves, San Salvador Island, Bahamas: in White, B., (ed.), Proceedings of the 6th Symposium on the Geology of the Bahamas, Bahamian Field Station,San Salvador, Bahamas: 17-21

BROCK T. D., MARTINKO, J. M., AND PARKER,J., (1996) Biology of Micro-organisms 8th ed. Prentice Hall, Englewood Cliffs, NJ

BROCKMAN, F. J., KIEFT, T.C., FREDRICKSON, J. K. BJORNSTAD, B.N., SPANGENBURG, S. W. AND LONG, P. E., (1992) Microbiology of vadose zone paleosols in south central Washington State: *Microbiol Ecology* **23**: 279-301

BUCHANAN, R. E. AND GIBBONS, N. E., eds. (1974) Bergy's Manual of Determinative Bacteriology, 8th ed. Williams and Wilkins, Baltimore MD.

BUCZYNSKI, C. AND CHAFETZ, H. S., (1990) Habit of bacterially induced precipitates of calcium carbonate and the influence of medium vicosity on mineralogy: *Journal Of Sedimentary Petrology* **61** (2) 226-233

BUKOWSKI, J.M., (1998) Modeling the fresh water-salt water interface in the Pleistocene aquifer on Andros Island, Bahamas, in: The 9th Symposium on the Geology of the Bahamas and other carbonate regions; Abstracts and Programs: Bahamian Field Station, San Salvador, Bahamas, p.8

BULLESBACH, E. E.AND SCHWABE, C., (1991) Total synthesis of human relaxin and human relaxin derivatives by solid-phase peptide synthesis and site-directed chain combination: *The Journal of Biological Chemistry* **266**: (17): 10754-10761

CAHILL, J. M., (1967) Hydraulic sand-model study of the cyclic flow of salt water in a coastal aquifer: *United States Geological Survey Professional Paper*, **575-B:** 240-244

CANT, R. V. AND WEECH, P. S., (1986) A review of the factors affecting the development of Ghyben-Hertzberg lenses in the Bahamas: *Journal of Hydrology* **84:** 333-343

CAPPENBERG, T. E. AND PRINS, R. A., (1974) Interrelationships between sulfate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake.III: Experiments with ^{14}C-labelled substrates. *Antonie van Leeuwenhoek* **40:** 457-469

CAREW, J.L. AND MYLROIE, J. E., (1992) Subaerial fossil reefs and phreatic dissolution caves: Indicators of Late Quaternary sea level and the tectonic stability of the Bahamas: *Geological Society of America Abstracts with Programs* **24**: (1) 6

CAVANAUGH, C. M., (1985) Symbiosis of Chemoautotrophic bacterial and marine invertebrates from hydrothermal vents and reducing sediments: *Biological Society of Washington Bulletin* **6**: 373-388

CHAPELLE, F. H. AND LOVELY, D. R., (1990) Rates of microbial metabolism in deep coastal plain aquifers: *Applied Environmental Microbiology* **56** 1865-1874.

CHAPELLE, F. H. AND LOVLEY, D. R., (1992) Competitive exclusion of sulfate reduction by Fe(III)-reducing bacteria: A mechanism for producing discrete zones of high-iron ground water: *Ground Water* **30**: 29-36

CHAPELLE, F. H., (1993) Ground-Water Microbiology and Geochemistry, 424 pp., John Wiley, New York

CHRISTENSEN, D., AND BLACKBURN,T. H., (1982) Turnover of ^{14}C-labelled acetate in marine sediments: *Marine Biology* **71**: 113-119

CLARKE, J. A., SARABIA, V., KEENLEYSIDE, W., MACLACHLAN, P.R.AND WHITFIELD, C., (1991) The compositional analysis of bacterial extracellular polysaccharides by high-performance anion exchange chromatography: *Anal. Biochemistry* 68-71

CLINE, J. D., (1969) Spectrophotometric determination of hydrogen sulfide in natural waters: *Limnology Oceanography*, **14**: 454-458

CLOUD, P. E., JR., AND LICARI, G. R., (1968) Microbiotas of the banded iron formations: *Proceedings of the National Academy of Science USA* **61**: 779-786

COLEMAN, M. L., (1985) Geochemistry of diagenetic non-silicate minerals: kinetic considerations: *Philosophical Transaction fo the Royal Society of London* **315**: 39-56

COLLERAN, E., (1992) Anaerobic digestion of agricultural and food-processing effluents in: Microbial control of pollution (J. C. Fry, G. M. Gadd, R. A. Herbert, C. W. Jones and I. A. Watson-Craik, eds., pp. 199-226. Cambridge University Press

COOPER, H.H., (1959) A hypothesis concerning the dynamic balance of freshwater and saltwater in a coastal aquifer: *Journal of Geophysical Research* **64:**461-467

COOPER, H. H., KOHOUT, F. A., HENRY, H. R. AND CLOVER, R. E., (1964) Sea water in coastal aquifers: United States Geological Survey, Water Supply Paper **1613-C** 84 p.

CRAGG, B. A., BALSE, S. J. AND PARKES, R. J., (1992) A novel method for the transport and long term storage of cultures and samples in an anaerobic atmosphere: *Letters in Applied Microbiology* **15:** 125-128

CRAGG, B. A. , HARVEY, S. M. , FRY, J. C., HERBERT, R. A., AND PARKES, R. J., (1992) 46. Bacterial biomass and activity in the deep sediment layers of the Japan sea, hole 798B: *Proceedings of the Ocean Drilling Program Scientific Results* **127/128** (1)

CRUEGER, W., AND CRUEGER, A., (1990) Biotechnology. Brock, T. D., (ed.), 2nd edition. Sinauer Associates, Sunderland MA.

DANDO, P. R., HUGHES, J. A., AND THIERMANN, F., (1995) Preliminary observations on biological communities at shallow hydrothermal vents in the Aegean Sea: *Hydrothermal Vents and Processes, Geological Society Special Publication* No. **87:** 303-317

DAVIS, R. L. AND JOHNSON, C. R., (1989) Karst hydrology of San Salvador, in Mylroie, J. E., ed., Proceedings of the fourth symposium on the geology of the Bahamas: Bahamian Field Station, Port Charlotte,Florida, pp. 118-135

DE ANGELIS, M. A., REYSENBACH, A. L. AND BAROSS, J. A.,(1991) Enhanced microbial methane oxidation in water from a deep-sea hydrothermal vent field at simulated *in situ* hydrostatic pressures: *Limnol. Oceanographic* **36:** (3) 565-570

DIETZ, R.S., HOLDEN,J.C. ANDSPROLL,W.P., (1970) Geotectonic evolution and subsidence of the Bahama Platform: *Geological Society of American Bulletin* **81**: 1915-1927

DIX, J. R., AND MULLINS, H.T., (1988) Rapid burial diagenesis of deep-water carbonates: Exuma Sound, Bahamas: *Geology* **16**: 680-683

DOMENICO, P. A. AND SCHWART, F. W., (1990) Physical and chemical hydrogeology; John Wiley & Sons Inc,. Canada.

EARDLEY, A. J., (1962) Structural geology of North America. 2Nd ed. New York: Harper & Row. 743p.

EATON, M.R., AND BOARDMAN, M.R., (1985) North African dust and its relation to paleoclimate recorded in a sediment core from northwest Providence Channel, Bahamas: *Geological Society of America Abstracts with Programs* **17**: p. 572.

EBERLI, G. P. AND GINSBERG, R. N., (1987) Sedimentation and coalesence of Cenozoic carbonate platforms, northwest Great Bahama Bank: *Geology* **5**: (1) 75-79

EDMUNDS, W. M AND WALTON, N. R. G., (1983) The Lincolnshire limestone-hydrochemical evolution over a ten year period: *Journal of Hydrology* **61**: 291-211

EGLINTON, G., PARKES, R. J., AND ZHAO, M., (1993) Lipid biomarkers in biogeochemistry: Future roles?: *Marine Geology* **113**: 141-145

EHRLICH, H. L., (1978) Inorganic energy sources for chemolithotrophic and mixotrophic bacterial: *Geomicrobiology Journal* **1**: 65-83

EHRLICH, H. L.,(1981) Geomicrobiology, Marcel Dekker, Inc., New York

EHRLICH, H. L., (1990) Geomicrobiology, second edition, Marcel Dekker, Inc., New York

EXLEY, S., (1986) Basic Cave Diving-a blueprint for survival; 5th edition, CDS-NSS, Inc.

FEIN, J. B., DAUGHNEY, C. J., YEE, N. AND DAVIS, T., (1997) The thermodynamics of metal adsorption onto bacterial surfaces. Geochim Cosmochim Acta

FENCHL, T. AND BLACKBURN, J. H., (1979) Bacteria and mineral cycling London: Academic Press

FENCHL, T. AND GLUD, R. N., (1998) Veil architecture in a sulphide-oxidizing bacterium enhances countercurrent flux: *Nature* **394**: 367-369

FERGUSON, J. AND LAMBERT, I. B. (1972) Volcanic exhalations and metal enrichments at Matupi Harbor, New Britain, T. P. N. G. *Economic Geology* **67**: 25-37

FERRIS, F. G., WIESE, R. G. AND FYFE, W. S., (1994) Precipitation of carbonate minerals by microorganisms: implications for silicate weathering and the global carbon dioxide budget: *Geomicrobiology Journal* **12**: 1-13

FLIERMANS, C. B., MCKINSEY P. C. AND FRANCK, M. M. (1993) *Abstract in the International Symposium on Subsurface Microbiology* **B34**

FORTIN, D., FERRIS, F. G. AND BEVERIDGE, T. J., (1997) Surface-mediated mineral development by bacteria in J.F. Banfield & K. H. Nealson eds., Geomicrobiology: Interactions betweeen microbes and minerals; Reviews in mineralogy **35** 161-180.

FOSSING, H. AND JØRGENSEN, B. B., (1989) Measurement of bacterial sulfate reduction in sediments: evaluation of a single-step chromium reduction method: *Biogeochemistry*, **8**: 205-222

FOWLER, C. M. R., (1990) The Solid Earth; Cambridge Univerity Press, 472p.

FRAZIER, W. J. AND SCHWIMMER, D.R., (1987) Regional stratigraphy of North America. New York: Plenum Press

FREDRICKSON, J. K., GARLAND, T. R., HICKS, R. J.,THOMAS, J. M., LI, S.W. AND MCFADDEN, S. M., (1989) Lithotrophic and heterotrophic bacteria in deep subsurface sediments and their relation to sediment properties: *Geomicrobiology Journal* **7**: 53-66

FREDRICKSON, J.K., CROCKMAN, F. J., WORKMAN, D.J., LI, S.W. AND STEVENS, T. O., (1991) Isolation and characterization of a subsurface bacterium capable of growth on toluene, naphthalene and other aromatic compounds: *Applied Environmental Microbiology* **57** 796-803.

FREDRICKSON, J.K., BROXKMAN, F. J., BJORNSTAD, B. N., LONG P. E., LI, S.W., MCKINLEY, J. P. WRIGHT, J. V., CONCA, J. L., KIEFT, T. L AND BALKWILL, D. L., (1993) Microbiological characteristics of pristine and contaminated deep vadose sediments from an arid region: *Geomicrobiology Journal* **11**: 95-107

FREDRICKSON, J.K., MCKINLEY, J. P., NIERZWECKI-BAUER,S A., WHITE, D. C., RINGELBERG, D. G., RAWSON,S. A., LI, S. W., BROCKMAN, F. J. AND BJORNSTAD, B. N., (1995) Microbial community structure and biogeochemistry of Miocene subsurface sediment: implications for long-term microbial survival: *Molecular Ecology* **4**: 619-626.

FREDRICKSON, J. K. AND ONSTOTT T.C. (1996) Microbes deep inside the earth: *Scientific American* : 68-73

FRICKE, H., GIERE, O., STETTER, K., ALFREDSSON, G. A., KRISTJANSSON, J. K., STOFFERS, P AND AVAVARSSON, J. (1989) Age of the formation of the Aegean active volcanic arc. in: Doumas, C. ed., Thera and the Aegean World II, Papers and Proceedings of the Second International Congress, Santorini, Greece. Thera and the Aegean World, London.

FRY, J. C., (1988) Determination of biomass. in Austin, B., ed., Methods in Aquatic Bacteriology: Chichester (Wiley), 27-72

FRY, J. C., (1990) Direct methods and biomass estimation: *Methods in Microbiology*, **22**: 41-85

FUHRMAN, J. A., AND AZAM, F., (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: Evaluation and field results: *Marine Biology* **66:** 109-120

FURNES, H., THORSETH, I. H., TUMYR, O., TORSVICK. T. AND FISK, M. R., (1996) 13. Microbial activity in the alteration of glass from pillow lavas from hole 896A[1]:*Proceedings of the ocean Drilling Program, Scientific Results* **148**:191-206.

GAFFEY, S. J., (1988) Water and organics in skeletal carbonates: Early diagenetic effects A progress report: in Mylroie, J. E., ed., Proceedings of the 4th Symposium on the Geology of the Bahamas, College Centre of the Finger Lakes, Bahamian Field Station, San Salvador, Bahamas: 145-157

GARRETT, P. AND GOULD, S.J., (1984) Geology of New Providence Island, Bahamas: *Geological Society of America Bulletin* **95**: 209-220

GARRISON, T., (1996) Oceanography, An invitation to marine science 2nd edition. New York: Wadsworth Pub. Co.

GETLIFF, J. M., (1991) Growth and productivity of shallow water and deep sea sediment bacteria [Ph.D. dissertation]. University of South Wales.

GHIORSE, W. C., (1997) Subterranean Life: *Science* **275**:789-790.

GHIORSE, W.C., AND WILSON, J.L., (1988) Microbial ecology of the terrestrial subsurface: *Advances in Applied Microbiology* **33**: 107-172

GIBLIN, A.E., (1988) Pyrite formation in marshes during early diagenesis: *Geomicrobiology Journal* **6**: 77-97

GIBSON, D. T. G., AND PARKINSON,D., (ed) (1984) Microbial degradation of organic compunds.New York: Marcel Decker Inc.

GLACCUM, R. A. AND PROSPERO, J. M., (1980) Saharan aerosols over the tropical North Atlantic-mineralogy: *Marine Geology:* **37** 295-321

GOLD, T., (1992) The deep, hot biosphere: *Proc. Natl. Acad. Sci. U.S.A.* **89**: 6045-6049

GOLUBIC, S. I. AND SCHNEIDER, J., (1979) Carbonate dissolution in: P. A. Trudinger and D.J. Swaine, eds., Biogeochemical Cycling of Mineral-Forming Elements. Elsevier, Amsterdam, pp. 107-129

GOULDER, R., (1977) Attached and free bacteria in an estuary with abundant suspended solids: *Journal of Applied Bacteriaology*, **43**: 399-405

GREENFIELD, L. J., (1963) Metabolism and concentration of calcium and magnesium and precipitation of calcium carbonate by a marine bacterium: *Ann. NY Acad. Sci.* **109**: 23-45

HAGSTROM, A., LARSSON, U., HORSTEDT, T. AND NORMARK, S., (1979) Frequency of dividing cells, a new approach to the determination of bacterial growth rates in aquatic environment: *Applied Environmental Microbiology*, **37**: 805-812

HALDERMAN, D. L. AND AMY, P. S., (1993) Bacterial heterogeneity in deep subsurface tunnels at Rainier Mesa, Nevada Test Site: *Microbial Ecology*, **25**: 183-194

HANSHAW, B. B. AND BACK, W., (1980) Chemical mass-wasting of the northern Yucatan Peninsula by groundwater dissolution: *Geology* **8**: 222-224

HARDIE, L. A. AND GINSBURG, R. M., (1977) Layering: The origin and environmental significance of lamination and thin bedding. in Hardie L. A. Ed., Sedimentation on the modern carbonate tidal flats of northwest Andros Island, Bahamas. John Hopkins University Studies in Geology 22, John Hopkins University Press, Baltimore, 50-123

HARIDON, S. L., REYSENBACH, A. L., GLENAT, P., PRIEUR, D.AND JEANTHON, C., (1995) Hot subterranean biosphere in a continental oil reservoir: *Nature* **377**: 223-224

HASHIMOTO, J., MIURA, T., FUJIKURA, K, AND OSSAKA, J. (1993) Discovery of vestimentiferan tube-worms in the euphotic zone: *Zoological Science* **10**: 1063-1067

ILIFFE, T. M., (1984) The zonation model for the evolution of aquatic faunas in `anchialine caves: *2560 Stygologia Schijf* : 312.

ILIFFE, T. M., (1986) Observations on the biology and geology of anchialine caves: in Curran, H. A., ed., Proceedings of the third Symposium on the Geology of the Bahamas, Bahamian Field Station,: 73-80

ILIFFE, T. M., (1992) Anchialine cave biology, Monografias Museo Nacional De Ciencias Naturales Consejo Superior De investigaciones Cientificas, ed., Camacho, A. I., 613-636

JACKSON, T. A., (1975) Humic matter in natural waters and sediments: *Soil Science* **119**: 56-64

JACQ, E., GEESEY, G. AND PRIEUR, D. (1987) Etude preliminaire des communautes bacteriennes d'un site hydrothermal cotier (White-Point, California, USA) *Vie Milieu* **37**: 59-66

JAMES, J. M.,ROGERS, P. AND SPATE, A. P., (1989) Genesis of the caves of the Nullarbor Plain, Australia, in: Proceedings of the 10th International Congress of Speleology, Budapest, p. 263-265.

JAMES, J. M., (1994) Microorganisms in Australian Caves and their influence on speleogenesis *in* Ira D. Sasowsky and Margaret V. Palmer eds., Breakthroughs in Karst Geomicrobology and Redox Geochemistry: Special Publications 1. Karst Water Institute, Charles Town, West Virginia 111 pg.

JAMES, J. M. AND ROGERS, P., (1994) The "Mysterious" calcite precipitating organism of the Nullarbor Caves, Australia *in* Ira D. Sasowsky and Margaret V. Palmer eds., Breakthroughs in Karst Geomicrobology and Redox Geochemistry: Special Publications 1. Karst Water Institute, Charles Town, West Virginia 111 pg.

JAMES, N. P AND CHOQUETTE, P.W., (1984) Diagenesis 9-Limestones, the meteoric diagenetic environment: *Geoscience Canada* **11:** 161-194

JAMES, N. P. AND CHOQUETTE, P. W., eds.,(1988) Paleokarst, Springer-Verlag, New York, 416 p.

JANNASCH, H. W., AND MOTTL, M.J., (1985) Geomicrobiology of Deep-Sea Hydrothermal Vents: *Science* **229**: 717-725

JENSEN P.R., GIBSON, R. A.,LITTLER, M. M. AND LITTLER, D. S., (1985) Photosynthesis and calcification in four deep-water *Halimeda* species (*Chlorophyceae, Caulerpales*): *Deep-Sea Research* **32:** 451-464

JONES, J., (1994) Amino Acid and Peptide Synthesis. ed., Stephen G. Davies, Alden Press., Oxford. p. 86.

JONG, E.W. DE., AND VRIND, J. P. M., (1997) Algal deposition of carbonate and silicates; in J. F., Banfield & K. H. Nealson, eds., Geomicrobiology: interactions between microbes and minerals; Reviews in Mineralogy 35 267-307.

JØRGENSEN, B. B., (1977) Bacterial sulfate reduction within reduced microniches of oxidixed marine sediments: *Marine Biology* **41:** 7-17

JØRGENSEN, B. B., (1978) A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. III. Estimation from chemical and bacteriological field data: *Geomicrobiology Journal,* **1:** 49-64

JØRGENSEN, B. B., (1982) Ecology of the bacteria of the sulphur cycle with special reference to anoxic-oxic interface environments: *Phil. Trans. R. Soc. Lond.* **B 298:** 543-561

JØRGENSEN, B. B., (1988) Ecology of the sulphur cycle: Oxidative pathways in sediments in Cole, J. A and Ferguson, S. J., (eds.) The society for General Microbiology, The Nitrogen and Sulphur Cycles. New York: Cambridge University Press

KAMENEV, G. M., FADEEV, V. I., SELIN, N. I., TARASOV, V. G. AND MALAKEHOV, V. V. (1993) Composition and distribution of macro- and meiobenthos around sublittoral hydrothermal vents in the Bay of Plenty, New Zealand: *New Zealand Journal of Marine and Freshwater Research* **27**: 407-418

KANE, T. C. SARBU, S. AND KINKLE, B. K., (1994) Chemoautotrophy: Methodological approaches, geological implications, and a case study from southern Romania *in* Ira D. Sasowsky and Margaret V. Palmer eds., Breakthroughs in Karst Geomicrobology and Redox Geochemistry: Special Publications 1. Karst Water Institute, Charles Town, West Virginia 111 pg.

KARL, D.M., WIRSEN, C. O. AND JANNASCH,H. W., (1980) Deep-sea primary production at the Galapagos hydrothermal vent: *Science* **207**: 1345-1347

KEETON, W. T., AND GOULD, J. L., (1986) Biological Science 4th edition, W.W. Norton and Company, NY.

KERR, R. A., (1997) Life goes to extremes in the deep earth and elsewhere?: *Science* **276**: 703-704

KIEFT, T. L., AMY, P. S., BROCKMAN, F. J., FREDRICKSON, J. K., BJORNSTAD, B. N., AND ROSACKER, L. L., (1993) Microbial abundance and activities in relation to water potential in the vadose zones of arid and semiarid sites: *Microbial Ecology*: **26**: 59-78

KIEFT, T. L., FREDRICKSON, J. K.,MCKINLEY, J. P., BJORNSTAD, B. N., RAWSON, S. A., PHELPS, T. J., BROCKMAN, F. J. AND PFIFFNER, S. M., (1995) Microbiological comparisons within and across contiguous lacustrine, paleosol, and fluvial subsurface sediments: *Applied Environmental Microbiology* **61**: 749-757

KING, G. M., (1991) Measurement of acetate concentrations in marine pore water by using an enzymatic approach: *Applied Environmental Microbiology*, **57**: 3476-3481

KIRCHMAN, D. L., (1993) Particulate detritus and bacteria in marine environments, in: Aquatic Microbiology: An Ecolgical Approach, ed., T. Ford, Blackwell, Oxford

KOHOUT, F. A., (1967) Groundwater flow and the geothermal regime of the Floridian Platau: *Transactions of the Gulf Coast Association Geological Society* **17**: 339-354

KOHOUT, F. A., HENRY H. R. AND BANKS, J. E., (1977) Hydrogeology related to the geothermal conditions of the Floridan Plateau. in Smith D.I. and Griffin, G.M., eds., The Geothermal Nature of the Floridan Plateau, Special Publication No. 21, 1-40

KORNICKER, L S. AND ILIFFE, T. M., (1998) Myodocopid Ostracoda (Halocypridina, Cladocopina) from Anchialine Caves in the Bahamas, Canary Islands and Mexico: *Smithsonian Contributions to Zoology* **599**

KRUMBEIN,W. E., (1974) On the precipitation of aragonite on the surface of marine bacteria: *Naturwissenschaften* **61**: 176-178

KRUMHOLZ, L. R. AND SUFLITA, J. M. (1996) Ecological interactions among sulfate-reducing and other bacteria livng in subsurface Cretaceous rocks: American Society for Microbiology Meetings, New Orleans, LA, N-56 in: eds., P. Amy and D. L.Haldeman, (1997) The Microbiology of the Terrestrial Deep Subsurface, Lewis Pub., New York

KUENEN, J. G. AND ROBERTSON, L. A., (1988) Ecology of nitrification and denitrifiction: in eds., Cole, J. A and Ferguson, S. J., The Nitrogen and Sulfur Cycles. The Society for General Microbiology Symposium 42, Cambridge University Press, p. 490

LAND, L. S., (1973) Holocene meteoric dolomitisation of Pleistocene limestones, north Jamaica: *Sedimentology*, **20**: 411-424

LEE, C., (1992) Control on organic carbon preservation: The use of stratified water bodies to compare intrinsic rates of decomposition in oxic and anoxic systems: *Geochimica et Cosmochimica Acta* **56**: 3323-3335

LEWIS, D. L., AND GATTIE, D. K.,(1990) Effects of cellular aggregation on the ecology of microorganisms, *ASM News* **56:** 263-268

LIBES, S. M.,(1992) An introduction to Marine Biogeochemistry, John Wiley and Sons, Inc., 734 p.

LONGMAN, M. W., (1980) Carbonate diagenetic textures from near-surface diagenetic environments: *Geological Society of America Bulletin,* **64:** 461-487

LOVLEY, D. R., (1987) Organic matter mineralization with the reduction of ferric Iron: A review: *Geomicrobiology Journal* **5** (3/4): 375-399

LOVELY, D. R., CHAPELLE, F. H. AND PHILLIPS, E. J. P., (1990) Recovery of Fe(III)-reducing bacteria from deeply buried sediments of theAtlantic Coastal Plain: *Geology,* **18:** 954-957

LOVLEY, D. R. AND CHAPELLE, F. H., (1995) Deep subsurface microbial processes: *Geophysics* **33:** 365-381

LUNDGREN, D. G. AND WILVER, M. (1980) Ore leaching by bacteria: *Annual Reviews of Microbiology* **34**: 263-283

LYNTS, G. W., (1970) Conceptual model of the Bahamian platform for the last 135 million years; *Nature* **225**: 1226-1228

MACKENZIE, W. S. AND ADAMS, A. E., (1984) Atlas of sedimentary rocks under the microscope. New York: John Wiley and Sons

MADIGAN, M. T., MARTINKO, J. M. AND PARKER, J., (1996) Brock Biology of Microorganisms eight edition, Prentice Hall International, Inc., 929

MADIGAN, M. T., MARTINKO, J. M., AND PARKER, J.(1997) Brock Biology of Micro-organisms, eighth international edition, Prentic Hall Inc., 982

MALFAIT, B. T. AND DINKELMAN, M.G., (1972) Circum-Caribbean tectonics and igneous activity and the evolution of the Caribbean plate: *Geological Society of America Bulletin* **83**: 251-272

MANN, C. J., (1986) Composition and origin of material in Pre-Columbian pottery, San Salvador Island, Bahamas: *Geoarchaeology:* **1** 183-194

MARDEN, P., HERMANSSON, M., AND KJELLEBERG, S., (1988) Incorporation of tritiated thymidine by marine bacterial isolates when undergoing a starvation survival response: *Archives on Microbiology* **149:** 427-432

MARTINEZ, J. D., JOHNSON, K. S. AND NEAL, J. T., (1998) Sinkholes in evaporite rocks: *American Scientist,* **86:** 38-51

MATTHEWS, R. K., (1974) A process approach to diagenesis of reefs and reef associated limestones. *Society of Economic Palaeontologists and Mineralogists Special Publication* **18:** 234-256

MCMAHON, P. B. AND CHAPELLE, F. H., (1991) Microbial production of organic acids in aquitard sediments and its role in aquifer geochemistry: *Nature* **349:** 233-235

MCNABB, J. F., AND DUNLAP, W. J., (1975) Subsurface biological activity in relation to ground water pollution: *Ground Water* **13**: 33-44

MEYERHOFF, A. A. AND HATTEN, C.W.,(1974) Bahamas salient of North America: Tectonic framework, stratigraphy and petroleum potential: *American Association of Petroleum Geologists Bulletin* **58**: 1201-1239

MEYERS, W.J., (1978) Carbonate cements: their distribution and interpretation in Mississippian limestones of southwestern New Mexico: *Sedimentology,* **25:** 371-400

MIWA, H., HIYAMA, C AND YAMAMOTO, M., (1985) High-performance liquid chromatography of short-and long-chain in fatty acids as 2-nitrophenylhydrazides: *Journal of Chromatography,* **321:** 165-174

MIWA, H., YAMAMOTO, M AND MOMOSE, T., (1980) Colorimetric detection and determination of carboxylic acids with 2-nitrophenylhydrazine hydrochloride: *Chemical Pharmaceutacal Bulletin,* **28:** 599-605

MONGER, H. C., DAUGHERTY, L. A. AND LINDEMANN, W. C., (1991) Microbial precipitation of pedogenic calcite: *Geology* **19**: 997-1000

MORIARTY, D.J. W., (1986) Measurement of bacterial growth rates in aquatic systems from rates of nucleic acid synthesis: *Advances in Microbial Ecology*, **9**: 245-292

MORIARTY, D. J. W., (1990) Techniques for estimating bacterial growth rates and production of biomass in aquatic environments: *Methods in Microbiology*, **22**: 211-234

MORIARTY, D. J. W. AND BELL, R. T., (1993) Bacterial growth and starvation in aquatic environments: in ed., S. Kjelleberg, Starvation in Bacteria, Plenum Press, New York: 25-53

MORITA, R. Y., (1992) Starvation-survival of heterotrophs in the marine environment: *Advances in Microbial Ecology* **6**: 117-198

MOSES, V. AND SPRINGHAM, D. G., (1982) Reservoir microbiology, in: V. Moses and D.G. Springham, eds., Bacteria and the enhancement of oil recovery: Applied Science Publishers Ltd., London

MUELLER-HARVEY, I. AND PARKES, R. J., (1987) Measurement of volatile fatty acids in pore water from marine sediments by HPLC: *Estuary Coastal Shelf Science*, **25**: 567-579

MULLINS, H. T. AND HINE, A. C., (1989) Scalloped bank margins: Beginning of the end for carbonate platforms?: *Geology* **17**: 30-33

MULLINS, H.T. AND LYNTS, G.W., (1977) Origin of the Northwestern Bahama Platform: Review and reinterpretation: *Geological Society of America Bulletin* **88**: 1147-1161

MURPHY, E. M., SCHRAMKE, J. A., FREDICKSON, J. K., BLEDSOE, H. W., FRANCES, A. J., SKLAREW, D. S. AND LINEHAN, J. C., (1992) The influence of microbial activity and sedimentary organic carbon on the isotope geochemistry of the Midendorf aquifer: *Water resour. Res.*, **28**: 723-740

MUSSMAN, W. J., MONTANEZ, I. P. AND READ, J. F., (1988) Ordovician Knox paleokarst unconformity Appalachians in James N. P. And Choquette, P. W.,eds., Paleokarst, Springer Verlag, New York, 211-228

MYLROIE, J. E. AND CAREW, J.L., (1990) The flank margin model for dissolution cave development in carbonate platforms: *Earth Surface Processes and Landforms* **15**: 413-424

NAGANUMA, T. (1991). Collection of chemosynthetic sulfur bacteria from a hydrothermal vent and submarine volcanic vents. in: Technical Reports Presented at the 7th Symposium on Deepsea Research Using the Submersible SHINKAI 2000; System. Japan Marine Science and Technology Center, Yokosuka 201-219.

NAZINA, T. N., ROZANOVA, E. P. AND KUZNETSOV, S. I., (1985) Microbial oil transformation processes accompanied by methane and hydrogen-sulfide formation: *Geomicrobiol. Journal* **4**: 103-130

NEALSON,K. H., (1983) The microbial iron cycle, p. 159-190. in W.E. Krumbein (ed.), Microbial Geochemistry. Blackwell, Oxford.

NEALSON, K. H., (1997) Sediment bacteria: Who's there, what are they doing, and what's new: *Annual reviews of Earth Planet* **25**: 403-434

NESSE, W. D., (1986) Introduction to Optical Mineralogy. New York: Oxford University Press

NEUMANN, C. A., BEBOUT, B. M., MCNEESE, L. R., PAULL, C. K. AND PAERL, H. A., (1988) Modern stromatolites and associated mates; San Salvador, Bahamas in John Mylroie ed.; Proceeding of the 4th Symposium on the Geology of the Bahamas: CCFL Bahamian Field Station, p. 235-251.

NILSEN, R. K., TORSVIK, T. AND LIEN, T., (1996) *Desulfotomaculum thermocisternum* sp. Nov., a Sulphate Reducer Isolated from a Hot North Sea Oil Reservoir: *Internation Journal of Systematic. Bacterology*, **46**: 397-402

NORTH, F. K., (1985) Petroleum Geology. Allen &Unwin, Boston.

NORTHROP, J. I., (1891) Notes on the geology of the Bahamas: *Transcripts of the New York Acadamy of Science* **10**: 4-23.

OBERDORFER, J. A., HOGAN, P. J AND BUDDEMEIR, R. W., (1990) Atoll island hydrology; flow and fresh water occurrence in a tidally dominated system: *Journal of Hydrology*, **120**: 327-340

OKI, D. S., SOUZA, W. R.,BOLKE, E. L, AND BAUER, G. R., (1998) Numerical analysis of the hydrogeologic controls in a layered coastal aquifer system, Oahu, Hawaii, USA: *Hydrogeology Journal* **6**: 243-263

OLAFSSON, J., THOR, K. AND CANN, J. (1991) A sudden cruise off island: *Ridge Events* **2**: 35-38

PAERL, H. W., (1978) Microbial organic carbon recovery in aquatic systems: *Limnol. Oceanograpy* **23**: 927-935

PAIN, S., (1998) The intraterrestrials: *New Scientist*: **2124**: 28-32

PAINE, S. G., LINGOOD, F. V., SCHIMMER, F. AND THRUPP, T. C., (1933) IV. The relationship of microorganisms to the decay of stone: *Royal Society of PhilosopicalTranslations*, **222B**: 97-127

PALMER, A.N., (1981) A Geological Guide to Mammoth Cave National Park, Zephyrus Press, Inc., p. 196

PALMER, R. J., (1985) The Blue Holes of the Bahamas, London, Jonathan Cape Ltd., 183

PALMER, R. J., (1994) An Introduction to Technical Diving; Underwater World Pub., London. p. 119

PALMER, R. J., (1995) Baha Mar, An underwater guide to the Bahamas, Immel Publishing Ltd. p. 208

PALMER, R.J., (1996) Blue Holes Foundation Newsletter Issue (2) P.O.Box F-44722, Freeport, Grand Bahama, The Bahamas

PALMER, R. J., (1997) Lusca's Breath. in DeepTech Advanced Diving Techniques. Winter ed.: 26-30

PALMER, R. J., WARNER, G. F.,CHAPMAN,P. AND TROTT, R. J., (1986) Habitat zonation in underwater caves in the Bahamas: *Proc. Int. Spelol. Cong.* 112-115

PALMER, R. J. AND WILLIAMS, D., (1984) Cave development under Andros Island, Bahamas: *Cave Science* **11:** 50-52

PARKES, R. J. AND BUCKINGHAM, W. J., (1986) The flow of organic carbon through aerobic respiration and sulfate-reduction in inshore marine sediments in: Perspectives in microbial ecology, F. Megusar and M. Gantar, eds., 4th International Symposium on Microbial Ecology, pp. 617-624

PARKES, R. J., CRAGG, B. A., BALE, S. J., GETLIFF, J. M., GOODMAN, K, ROCHELLE, P. A., FRY, J. C., WEIGHTMAN, A. J., AND HARVEY, S. M., (1994) Deep bacterial biosphere in Pacific Ocean sediments: *Nature* **371**: 410-413

PARKES, R. J., GIBSON, G. R., MUELLER-HARVEY, L., BUCKINGHAM, W. J. AND HERBERT, R. A., (1989) Determination of the substrates for sulfate-reducing bacteria within marine and estuarine sediments with different rates of sulphate reduction: *Journal of General Microbiology* **135**: 175-187

PEDERSEN, K. AND EKENDAHL, S., (1990) Distribution and activity of bacteria in deep granitic groundwaters of southeastern Sweden: *Microbial Ecology* **20**:37- 52

PEDERSEN, K., EKENDAHL, S., TULLBORG, E. L., FURNES, H., THORSETH, I. AND TUMYR, O., (1997) Evidence of ancient life at 207 m depth in a granitic aquifer: *Geology,* **25:** (9) 827-830

PENTECOST, A., (1990) Obervations on the Scytonema mats of San Salvador, Bahamas: in Myroie, J. E., ed., Proceeding of the 4th Symposium on the Geology of the Bahamas, Bahamian Field Station,: 295-302

PROSSER, J. AND GREY, H.V., (eds.), (1992) NSS Cave Diving Manual: An Overview, CDS-NSS Pub., P.O.Box950 Branford Florida 32008-0950 U.S.A. p. 377

RAMSING, N. B., FOSSING, H. FERDELMAN, T. G., ANDERSEN, F. AND THAMDRUP, B., (1996) Distribution of bacterial populations in a stratified fjord (Mariager Fjord, Denmark) Quantified by *in situ* hybridization and related to chemical gradients in the water column: *Applied and Environmental Microbiology*, **62:** (4) 1391-1404

REPETA, D. J., SIMPSON, D.J., JØRGENSEN, B. B. AND JANNASCH, H. W., (1989) Evidence for anoxygenic photosynthesis from the distribution of bacteriochlorophylls in the Black Sea: *Nature*, **342:** 69-72

ROSNES, J. T., TORSVIK, T. AND LIEN, T., (1991) Spore-forming thermophilic sulfate-reducing bacteria isolated from North Sea oilfield waters: *Applied Environmental Microbiology*, **57:** 2302-2307

ROZANOVA, E. P., (1978) Sulfate reduction and water-soluble organic substances in a flooded oil reservoir: Mikrobiologiya **47** : 495-500 (engl. transl. pp 401-405)

RUETER, P., RABUS, R., WILKES, H., AECKERSBERG, F., RAINEY, F. A.,JANNASCH, H. W. AND WIDDEL, F., (1994) Anaerobic oxidation of hydrocarbons in crude oil by new types of sulphate-reducing bacteria: *Nature* **372**: 455-458

SARANO, F., MURPHY, R. C., HOUGHTON, B. F., AND HEDENQUIST, J. W. (1989) Preliminary observations of submarine geothermal activity in the vicinity of White Island Volcano, Taupo Volcanic Zone, New Zealand: *Journal of the Royal Society of New Zealand* **19**: 449-459

SARBU, S. M. AND POPA, R., (1992) A unique chemoautotrophically based cave ecosystem: *The Natural History of Biospeleology* **1**: 638-666

SCHLAGER, W. AND GINSBURY, R. N., (1981) Bahama carbonate platforms-the deep and past: *Marine Geology* **44**: 1-24

SCHWABE, S. J. AND WHEELER, F., (1998) Sterile and general sampling techniques for submerged cave environments: *Underwater Scientific Technology* (in press)

SCHWABE, S. J., CAREW, J. L. AND MYLROIE, J. E.,(1993) The petrology of Bahamian Pleistocene eolianites and flank-margin caves: Implication for Late Quaternary island development, in White, B., ed., Proceedings of the Sixth Symposium on the Geology of the Bahamas, San Salvador, Bahamas, Bahamian Field Station,:149-164.

SEALEY, N. E., (1994) Bahamian Landscapes, An Introduction to the Geography of the Bahamas, 2nd edition, Media Publishing p. 128.

SHAW, T., (1993) The history of cave studies in Trinidad, America, the Bahamas and some other caribbean islands: *ACTA Carsological* **22**: 11-76

SHERIDAN R. E., CROSBY, J.T., BRYAN, G. M. AND STUFFA, P. L.,(1981) Stratigraphy and structure of southern Blake Plateau, northern Florida Straits and northern Bahama Platform from multichannel reflection data: *American Association of Petroleum Geologist Bulletin* **65**: (12) 2571-2593

SHEVENELL, L AND GOLDSTRAND, M, P., (1997) Geochemical and depth controls on microporosity and cavity development in the Maynardville Limestone: Implication for groundwater: *Cave and Karst Science* **24** (3) 127-136.

SHI, Y., ZWOLINSKI,M. D., SCHREIBER, M.E. , BAHR, J. M., SEWELL, G. W. AND HICKEY, W. J., (1999) Molecular Analysis of Microial Community Structures in Pristine and Contaminated Aquifers: Field and Laboratory Microcosm Experisments: *Applied and Environmental Microbiology*, (65) **5:** 2143-2150.

SHIVELY, J. M AND BARTON, L. L.,(1991) Variations in Autrotrophic Life. London: Academic Press

SIMON, M., (1985) Specific uptake rates of amino acids by attached and free-living bacteria in a mesotrophic lake: *Applied Environmental Microbiology*, **49:** 1254- 1259

SINCLAIR, J. L. AND GHIORSE, W. C. (1989) Distribution of aerobic bacteria, protozoa, algae, and fungi in deep subsurface sediments: *Geomicrobiology Journal* **7**: 15-32

SMART, P. L., DAWANS, J.M. & WHITAKER, F., (1988) Carbonate dissolution in a modern mixing zone: *Nature* **335**: 811-817

SMART, P. L. AND WHITAKER, F. F., (1988) Controls on the rate and distribution of carbonate bedrock dissolution in the Bahamas. in Mylroie, J, ed., Proceedings of the 4th Symposium of the Geology of the Bahamas, Fort Lauderdale, College Center of the Finger Lakes Bahamian Field Station, San Salvador, Bahamas: 313-322

SØRENSEN, J., CHRISTENSEN, D. AND JØRGENSEN, B. B., (1981) Volatile fatty acids and hydrogen as substrates for sulfate-reducing bacteria in anaerobic marine sediment: *Applied Environmental Microbiology*, **42**: 5-11

SOROKIN, D. YU (1991) [Oxidation of reduced sulphur compounds in volcanic regions in the Bay of Plenty (New Zealand) and Matupy Harbour (New Britain, Papua-New Guinea).: *Proceedings of the USSR Academy of Science, Series Biological* **3**: 376-387 [In Russian]

SOROKIN, Y. I., (1970) Interrelations between sulphur and carbon turnover in meromictic lakes: *Arch. Hydrobiol.* **66**/4 :391-446

SPENCER, M., (1997) MYSTERIOUS McCAVITY: *Australian Geographic* **45** 48-63.

STANIER, R. Y., INGRAHAM, J. L., WHEELIS, M. C. AND PAINTER, P. R., (1986) The microbial world; Prentie-Hall, Englewood Cliffs, NJ.

STEIN, J. L.(1984) Subtidal gastropods consume sulphur-oxidising bacteria: evidence from coastal hydrothermal vents: *Science* **223**: 696-698

STETTER, K. O., BLOCHL, E, KURR, M., EDEN, R. E., FIELDER, M., CASH, H. AND VANCE, I., (1993) Hyperthermophilic archaea are thriving in deep North Sea and Alaskan oil reservoris: *Nature*, **365**: 743-745

STEVENS,T. O., MCKINLEY, J. P. AND FREDRICKSON, J. K., (1993) Bacteria associated with deep , alkaline, anaerobic groundwaters in southeast Washington: *Microbiol Ecology,* **25**: 35-50

STEVENS, T. O. AND MCKINLEY, J. P., (1995) Lithoautotrophic microbial ecosystems in deep basaltic aquifers: *Science* **270:** 450-454

STOESSELL, R. K., WARD, W. C., FORD, B. H. AND SCHUFFERT, J. D., (1989) Water chemistry and $CaCO_3$ dissolution in the saline portion of an open-flow mixing zone, coastal Yucatan Peninsula, Mexico*: Geological Society of America Bulletin,* **101:** 159-169

STRYER, L., (1981) Biochemistry, 2nd ed., W. H. Freeman and Co., San Francisco., p. 949

STUMM, W., (1992) Chemistry of the solid-water interface. New York: John Wiley and Sons, Inc

TARASOV, V. G., PROPP, M. V., PROPP, L. N., ZHIRMUNSKY, A. V., NAMSARAEV, B. B., GORLENKO, V. M. AND STARYNIN, D. A. (1990) Shallow-water gasohydrothermal vents of Ushishir volcano and the ecosystem of Kraternaya Bight (The Kuril Islands.): *Marine Ecology* **11:** 1-23

TARBOX, D. L., (1987) Groundwater occurrence and development in coastal karst terrains of oceanic islands in the lower latitudes, in Beck, B. F and Wilson, W L., eds., Karst Hydrology, Engineering and Environmental Applications: Florida Sinkhole Research Institute, pp. 287-290

TESKE, A., WAWER, C., MUYZER, G. AND RAMSING, N.B., (1996) Distribution of sulfate-reducing bacteria in a stratified fjord (Mariager Fjord, Denmark) as evaluated by most-probable-number counts and denaturing gradient gel electrophoresis of PCR-amplified ribosomal DNA fragments*:* *Applied and Environmental Microbiology* **62** (4) 1405-1415

THOMPSON,J.B., SCHULTZE-LAM, S., BEVERIDGE, T. J. DES MARAIS, D. J. (1997) Whiting events: biogenic origin due to the photosynthetic activity of cyanobacterial picoplankton: *Limnol Oceanogr* **42** 133-141.

TRAGER, G. C AND DENIRO, M. J (1990) Chemoautotrophic sulfur bacteria as a food source for mollusks at intertidal hydrothermal vents: evidence from stabel isotopes *Veliger* **33**: 359-362

TUCKER, M. E. AND WRIGHT, V. P., (1990) Carbonate Sedimentology. Blackwell, London, 482 p.

TUTTLE, J. H., WIRSEN, C. O. AND JANNASCH, H. W., (1983) Microbial activities in the emitted hydrothermal waters of the Galapagos Rift Vents: *Marine Biology* **73**: 293-299

VACHER, H. L., (1988) Dupuit-Ghyben-Herzberg analysis of strip-island lenses: *Geological Society of America Bulletin* **100:** 580-591

VACHER, H. L. AND HEARTY, P., (1989) History of stage 5 sea level in Bermuda: Review with new evidence of a brief rise to present sea level during substage 5a: *Quaternary Science Review,* **8:** 159-168

VACHER, H. L. AND BENGTSSTON, T. D., (1990) Effect of hydraulic conductivity on the residence time of metoric ground water in Bermudian and Bahamian type island, in Mylroie, J. E., ed., Proceedings of the 4th symposium on the geology of the Bahamas: Bahamaian Field Station, pp. 337-351

VERNON, R. O., (1969) The geology and hydrology associated with a zone of high permeability ('Boulder Zone') in Florida Society of Mining Engineers, American Institute of Mining Engineers, 69-AG-12

VIDAL, V. M., VIDAL, F. M. AND ISAACS, J. D. (1978) Coastal submarine hydrothermal activity off northern Baja California: *Journal of Geophysical Research* **83:** 1757-1774

WATKINSON,R. J. (ed) (1978) Developments in Biodegradation of Hydrocarbons. London: Applied Science Publisher, Ltd.

WELLSBURY AND PARKES, J., (1995) Acetate bioavailability and turnover in an estuarine sediment: *FEMS Microbiology Ecology* **620**

WELLSBURY, P., GOODMAN, K., BARTH, T., CRAGG, B., BARNES, S. P., AND PARKES, R. J., (1997) Deep marine biosphere fuelled by increasing organic matter availability during burial and heating: *Nature* **388**: 573-576

WEST, J. M., MCKINLEY, I. J., AND VIALTA, A., (1992) Microbiological analysis at the Pocos de Caldas natural analogue study sites: *Journal of Geochemical Explorer* **45**: 439-449

WHEATCRAFT, S. W. AND BUDDEMEIR, R. W., (1981) Atoll island hydrology: *Groundwater*, **19**: 311-320

WHITAKER, F.F., (1992) Hydrology, Geochemistry and Diagenesis of Modern Carbonate Platforms in the Bahamas: (Doctorial Thesis): Bristol University, Bristol, United Kingdom, 347 p.

WHITAKER, F. F. AND SMART, P. L., (1997) Groundwater circulation and geochemistry of karstified bank-marginal fracture system, South Andros Island, Bahamas: Journal of Hydrology **197**: 293-315

WHITE, W. B., (1988) Geomorphology and Hydrology of Karst Terrains. Oxford University Press, New York., p. 464

WIDDEL, F., SCHNELL, S., HEISING, S., EHRENREICH, A., ASSMUS, B. AND SCHINK, B., (1993) Ferrous iron oxidation by anoxygenic phototrophic bacteria: *Nature*, **362**: 834-836

WILSON, E. K., (1997) Discovering Other Worldly Life; Science & Technology: *C&EN* March 34-35

WOLFE, R S. AND HIGGINS, I. J., (1979) Microbial biochemistry of methane-a study of contrasts. in: International review of biochemistry, vol. 21. pp 260-280. Ed., Quale, J. R. Baltimore: University Park Press

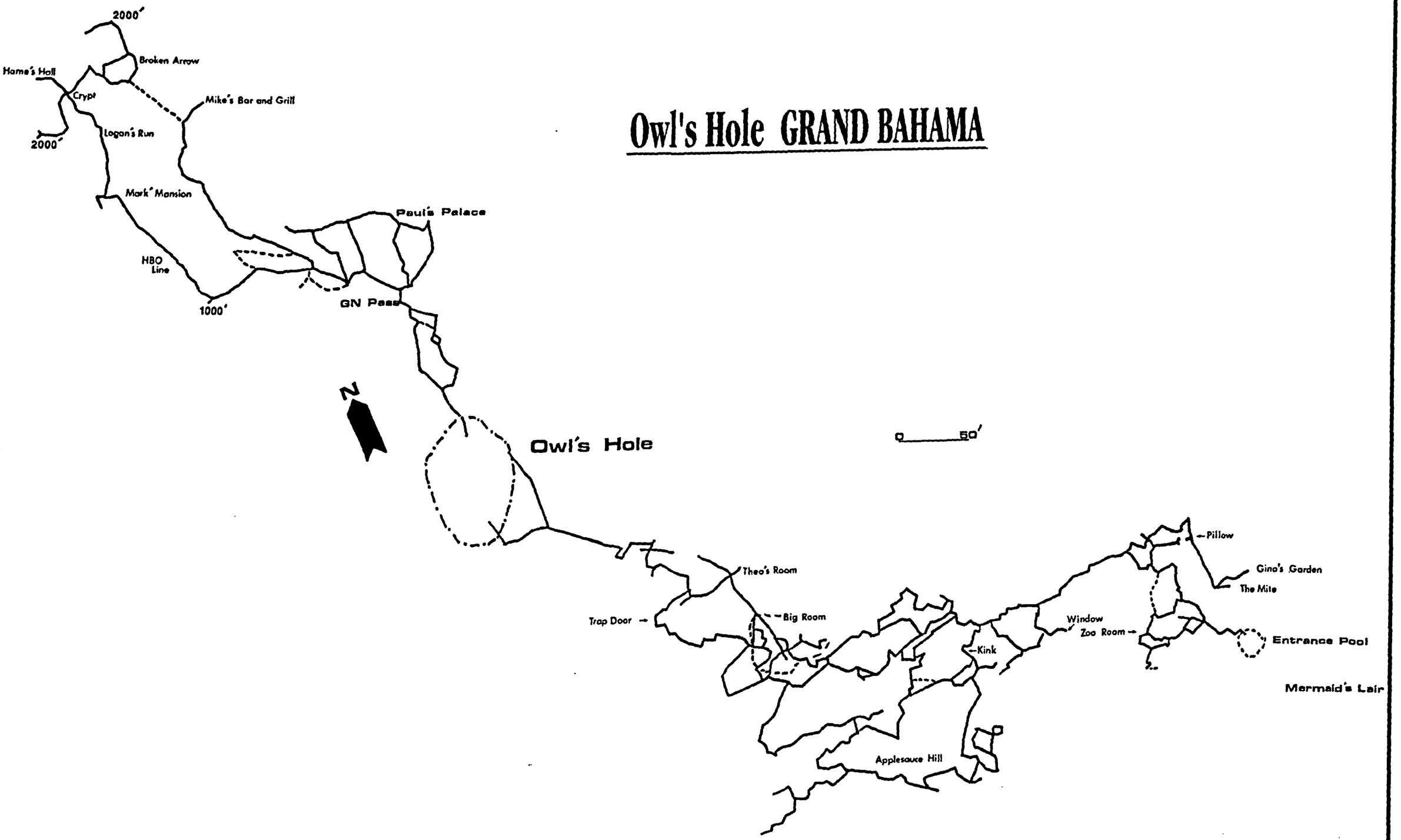
YAGER, J., (1981) Remipedia, a new class of Crustacea from a marine cave in the Bahamas: *Journal of Crustacean Biology* **1**: 328-333.

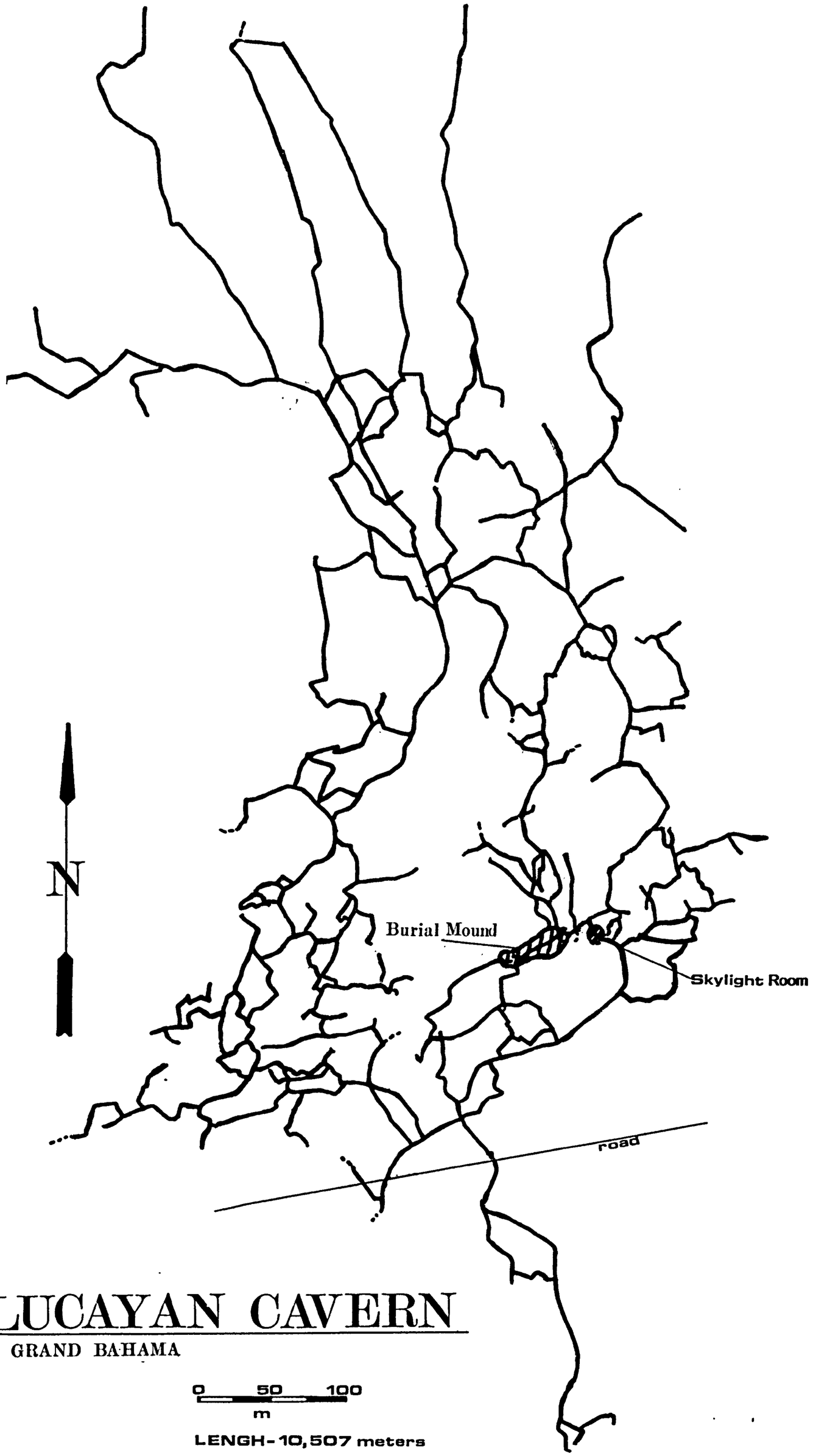
YARBROUGH, H. F.AND COTY, V. F.,(1983) Microbially enhanced oil recovery from the Upper Cretaceous Nacatoch formation, Union County, Arkansas: in Donaldson E.C. and Clark, J. B., (eds) Proceedings on the International Conference on Microbiol Enhancement Oil Recovery (Conf. -8205140). NTIS, Springfield, VA, pp.149-153

ZEHENDER, A. J. B., ed., (1988) Biology of Anaerobic Microorganisms, 872 pp., John Wiley, New York

ZOBELL, C. E., (1952) Part played by bacteria in petroleum formation: *Journal of Sedimentary Petrology* **22**:42-49

Owl's Hole GRAND BAHAMA





LUCAYAN CAVERN

GRAND BAHAMA

0 50 100
m

LENGH-10,507 meters

Appendix Three

Nitrox:

The primary use of enriched air Nitrox (EAN) in diving is to reduce the amount of nitrogen or inert gas present during the course of a dive and thus to reduce the potential for decompression illness and, to a degree, nitrogen narcosis. Side benefits can include a reduction interface interval for repetitive dives, and reduced level of subclinical decompression illness following a dive as well as longer "no-decompression" dive times. Richer mixtures are occasionally used for decompression from deep dives to reduce the inert gas gradient and so hasten the removal of inert gas (e.g. nitrogen) from the body tissues (Palmer 1997).

The average depth of our dives on Grand Bahama (Lucayan Caverns and Owl's Hole) ranged between 20-30 metres. Based on the safety principle, i.e., not to exceed 1.6 bar of PO_2, the ideal mix was Nitrox 36; a mixture containing 36% oxygen and 64% highly filtered compressed air. The air fraction had to be very clean (void of all hydrocarbons, lint etc.) because of the volatile nature of pure oxygen. Pure medical grade oxygen was decanted into an empty scuba cylinder first, followed by air. The mixture was than tested for oxygen percent using an oxygen sensor. The measured value was than written on a label on the cylinder. A variation of 2 to 3% was acceptable.

Rob Palmer Blue Holes Foundation and Cave Diving Policy and Standards for Conservation and Safety:

The RPBHF believes that caves have unique scientific, recreational, and scenic value: That these values are endangered by both carelessness and intentional vandalism: That these values once gone, cannot be recovered: and that the responsibility for protecting caves must be assumed by those who study and enjoy them.

Accordingly, the intention of the foundation is to work for the preservation of caves with a realistic policy supported by effective programs for: the encouragement of self-discipline among cavers; education and research concerning the causes and prevention of cave damage; and special projects, including cooperation with other groups similarly dedicated to the conservation of natural areas specifically: all contents of a cave-formations, life, and loose deposits-are significant for its enjoyment and interpretation. Therefore, caving parties should leave a cave as they find it. They should provide means for the removal of waste; limit marking to a few small and

removable signs as are needed for surveys; and , especially, exercise extreme care not to accidentally break or soil formation, disturb life forms or unnecessary increase the number of disfiguring paths through an area.

The foundation encourages projects such as: establish cave preserves; placing entrance gates where appropriate: opposing the sale of speleothems; supporting effect protective measures; cleaning and restoring over-used caves; cooperating with private cave owners by providing knowledge about their cave and assisting them in protecting their cave and property from damage during cave visits, and encouraging commercial cave owners to make use of their opportunity to aid the public in understanding caves and the importance of their conservation.

Where there is reason to believe that publication of cave locations will lead to vandalism before adequate protection can be established, the foundation will oppose such publications.

It is the duty of every trained cave diver to take personal responsibility for spreading a consciousness of the cave conservation problem to each potential user of caves. Without this, the beauty and value of our caves will not long remain with us.

For this project, cave diving safety policy which required that all members of the scientific team who enter cave environments be cave-certified, use a continuous guideline from the entrance, reserve 2/3 of air supplies for the exit from the cave, carry 3 lights one of which must contain disposable batteries, were followed to the letter following the first field trip. Scientific collection for this project did not damage the cave and permits are issued and current for all field excursions.